PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

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CONTENTS OF VOLUME XX

NUMBER 1

Temperature Effect upon Rate of Feeding in a Folliculinid . . . . . *E. A. Andrews* 1

The Effect of Certain Deficient Media on Resting Cyst Formation in *Colpoda duodenaria* *Laura Garnjobst* 5

Some Interrelations of Drug and Ion Actions in the Artificial Activation of Marine Eggs *Bradley T. Scheer and Marlin Ann Ray Scheer* 15

The Effects of Certain Denatured Organs on Neuralization in *Amblystoma* Explants *C. D. Van Cleave* 32

The Postnatal Development of the Reproductive System of the Golden Hamster (*Cricetus auratus*) and Its Reactivity to Hormones . . . . . *Evelina Ortiz* 45

Interrelations of Thyroid and Gonad in the Development of Plumage and Other Sex Characters in Brown Leghorn Roosters . . . . . *Ben B. Blivaiss* 67

New Books in Physiological Zoölogy 108

Harold Burrows, Biological Actions of Sex Hormones, *C. Donnell Turner.*—E. S. Russell, The Directiveness of Organic Activities, *W. C. Allee.*—Jean Brachet, Embryologie chimique, *L. G. Barth.*—Leo Loeb, The Biological Basis of Individuality, *R. W. Gerard.*

NUMBER 2

Plumage Sequence in a Case of Hemilateral Asymmetry in Male Hybrid Fowl *Mary Juhn* 117

The Relation of the Plumage-Tract Gradients to the Serial Localization of Feather Pigments in a Hybrid Cock . . . . . *Mary Juhn and Carl W. Hess* 125

Phosphorylation in the Frog's Egg . . . . . *L. G. Barth and L. Jaeger* 133

Riboflavin and Other Fluorescent Compounds in a Developing Egg (Orthoptera) *Joseph Hall Bodine and Laurence R. Fitzgerald* 146

The Effect of Thiourea on the Oxygen Consumption of *Fundulus* *Samuel A. Matthews and Dietrich C. Smith* 161

The Effect of Salts and Osmotic Concentration on the Size of Food Vacuoles in *Vorticella similis* . . . . . *W. J. Bowen* 165

An Analysis of Photoelectric Instruments for Measurement of Turbidity with Reference to Serology . . . . . *Joseph G. Baier, Jr.* 172

The Influence of the Sinus Glands upon Molting and Associated Changes in the Crayfish *Harold H. Scudamore* 187

New Books in Physiological Zoölogy 209

R. W. Moncrieff, The Chemical Senses, *Theodore Louis Jahn.*—M. J. D. White, Animal Cytology and Evolution, *C. L. Turner.*

Notice to Contributors 212

NUMBER 3

An Analysis of the Factors Influencing Growth and Development of the Mammalian Reproductive Tract . . . . . *Dorothy Price* 213

Origin of Pigment Cells from the Neural Crest in the Mouse Embryo *Mary E. Rawles* 248

Some Relations between Territory, Social Hierarchy, and Leadership in the Green Sunfish (*Lepomis cyanellus*) . . . . . *Bernard Greenberg* 267

Ovarian Activity in Mammals Subsequent to Chemical Injury of Cortex *Carl R. Moore and Hsi Wang* 300

New Books in Physiological Zoölogy 322
D. E. Lea, Actions of Radiations on Living Cells, *Raymond E. Zirkle.*

Notice to Contributors 324

NUMBER 4

The Effect of Temperature on the Intersex Character of *Daphnia longispina* *Katherine K. Sanford* 325

The Effect of Certain Depressing Agents on Intracellular Oxidation of Indicators in Hydra *C. M. Child* 332

Responses of Fish Melanophores to Chemical Warfare Agents
C. Ladd Prosser, Bruno von Limbach, and George W. Benhett with Technical Assistance of Marian F. James 349

Effects of Testosterone Propionate on Sex Development in Female *Ambystoma* Larvae *Beatrice Mintz* 355

The Effect of Male Hormone on Aggressive Behavior in Mice . . *Elizabeth A. Beeman* 373

The Transmission of Radio-Strontium and Plutonium from Mother to Offspring in Laboratory Animals . . . . . *Miriam P. Finkel* 405

New Books in Physiological Zoölogy 422
Theodor von Brand, Anaerobiosis in Invertebrates, *William Balamuth.*—A. Szent-Györgyi, Chemistry of Muscular Contraction, *C. Ladd Prosser.*

Index 427

PHYSIOLOGICAL ZOÖLOGY

Volume XX JANUARY 1947 *Number* 1

TEMPERATURE EFFECT UPON RATE OF FEEDING IN A FOLLICULINID

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THE folliculinids are ciliates, chiefly marine, differing from the fresh-water stentors in the greater development of the food-getting apparatus and in dwelling much of the time within bottle-shaped chitenoid tests.

A common folliculinid along both the North Atlantic coasts is *Parafolliculina amphora* Dons. The test it makes is shaped like a flat pocket flask with a short upturned neck and is about 200 μ long. The cytostome, or stoma, is at the bottom of a deep funnel.

When the food consists of innumerable minute particles, such as bacteria, ingestion commonly takes place as follows: the particles gradually accumulate at the bottom of a funnel whose floor distends to become a food sack about a spheroidal mass of food, and this sack with food is pinched off and swallowed.

This filled sack is the future food vacuole subject to digestion and is ultimately emptied out at the anus, which in this species is situated on the outer face of the middle of the left food-collecting lobe of the peristome. The pinching-off is instantaneous, and then the food passes for a few seconds through the passage in the endoplasm known to Moebius (1887), Sahrhage (1916), and Andrews (1914) as the "esophagus," to be slowly circulated then in the general endoplasm.

The pinching-off is the act of ingestion in which the food ceases to be outside the animal and gets within it, though always separated from the actual protoplasm by the vacuole wall which was the bottom of the funnel and which remains as the food vacuole wall until its contents are thrown out at the anus.

The intervals between successive pinchings-off of vacuoles is the rate of ingestion.

Most favorable views of food-taking are side views of the animal when expanded out of its test, but this occurs at irregular intervals, the animal slowly expanding, often with interruptions, and instantly jerking back again. Very many of the instantaneous withdrawals are referable to unknown internal states. One animal withdrew at the following intervals: 35, 12, 4, 20, and 20 seconds and another at intervals of 3, 5, 5, 4, and 3 seconds.

When the animal withdraws into its test, the funnel is pulled down along the mid-line and then bent to the left, so so that the stoma comes to lie far from

the mid-line and very near to the ectoplasm of the left side.

Though food-getting sometimes occurs when the peristomial lobes are within the test, this is seldom the case; but pinching-off does take place not only when the expanded animal carries its stoma outside the mouth of the test but also when the stoma is at any part of the course of the funnel bottom within the test, that is, all along the mid-line and far to the left side. Thus filling of the food sack is not always immediately followed by pinching-off, since, when down inside the test, a food sack may be brought out to the exterior to receive more food before being pinched off or it may be pinched off *when the time comes*, without being carried out again to be filled.

Moreover, the size of the food sack is not what determines its cutting-off, for there are both larger and smaller sacks cut off in a series in which most are of about the same bulk, without time being correlated with size.

To determine the intervals of ingestion with no disturbance, animals attached to slides submerged in 50–60 cc. water in a Petri dish were observed from above with Zeiss D* water-immersion and X Spencer ocular.

A specimen watched for nearly an hour and a half formed 42 food sacks at intervals of about 2 minutes, with a range of a rare 1 minute to $3\frac{1}{2}$ minutes: an average of $2\frac{1}{4}$ minutes. Of that series of 42, an interval of less than 2 minutes occurred but six times, and an interval of more than 2 minutes but nine times. Of the 42, 16 took place within the test.

On different days different animals gave the same indication of about 2 minutes as the general interval of ingestion.

More precisely recorded in seconds, there were such readings as the following (where the size is sometimes indicated, as well as whether cutoffs were within the test): 130, 122, 130? (in), 117? (in), 118, 130?, 354 (in), 122, 120, 123 (very small), 135, 173 (in), small one not seen in time (in), 253, 118, 132, 108, 110, 103 (large), 109 (large), 136 (in), 120 plus, 360 plus (in), 218, 90?, 129? (in), 120 plus (in), 103?, 150?, 180, 120 plus (in), 270 plus, 109 (in), 108 (in small).

In this record the five placed at 200 or over were instances in which a cutoff was overlooked; that is, the interval recorded is really two intervals. This would make the entire number of intervals 38, with an average of 129 and a range of 90–180, but with the most frequent intervals 110–30. Some seven readings were of doubtful accuracy.

Another reading of 16 intervals gave an average of 128, with a range of 120–38, as follows: 126, 128, 138, 135, 125, 125, 120, 135, 128, 120, 120, 128, 139, 120, 123, 135. Another gave 15 readings from 120 to 180, with an average of 143. Still another gave 13 readings from 60 to 147, averaging 111, with only two cutoffs inside the test.

When the laboratory was unusually warm, namely, 26°.5 C., the ingestion intervals were noticeably shorter, namely, as follows: 64, 120, 90, 75, 67, 75, 115, 62, 90, 70, 97, 88, 100, 75, 85, 106, 118, 93. The average was but 88, with a range of 64–118 (omitting two records exceeding 200). Another animal, on the same slide, gave 10 readings from 60 to 148, with an average of 97.7.

Then two animals, A and B, were cooled and heated to see the effect of temperature on each individual, with the results shown in Table 1.

In different trials the animals were exposed for different periods to the temperatures read. In general, an increase of about 10° C. was followed by twice the speed of ingestion!

Metalnikow (1912) found that in

paramecium the ingestion was influenced by very many factors, including temperature. But he did not observe actual ingestion processes but only their result; that is, he counted the numbers of food vacuoles present after half an hour of feeding. This number was different in different individuals; thus at 10° C. one might take only one food vacuole and another might take five, with an average for twenty of 2.5. However, at 20° C., the range in numbers taken was 4–8, and the average for twenty was 6.7. Again at 30° C., the range was 9–20, and the average for twenty was 14.4. Metalnikow was interested to note that the increase in numbers of food vacuoles taken was roughly double with each increase in temperature of 10° C, approximating the Van't Hoff law. However, some of his experiments were not so satisfactory from this point of view, though they all agree that an increase of temperature results in a greater number of food vacuoles within a given time.

Comparing the temperature effect observed by Metalnikow on the rate in paramecium with what was observed in *P. amphora*, if we assume that the food vacuoles were taken in at regular intervals, then the temperature rise from 20° to 30° speeded up the food-taking 2.16 times in paramecium. And in *P. amphora* the rise from 14°.5–19° to 25°.5–29° increased the speed 2.24 times. In both species a rise of about 10° is followed by more than double speed.

Although under some circumstances the food vacuoles in *P. amphora* may be ingested with a regular rhythm, this is but one aspect of the phenomena of ingestion, and much food is taken otherwise; for, as in the folliculinids studied by Moebius (1887), the stomal film may not be cut off to accompany the food down the esophagus but may open at its bottom, as seen by Moebius, and then the food mass is conducted through the esophagus and into the endoplasm to acquire a food vacuole wall later, by action of the endoplasm. This swallowing without food sack is interpreted as a pinching-off not at the upper end of the food sack but lower down.

TABLE 1

INGESTION INTERVALS IN *P. amphora* AT DIFFERENT TEMPERATURES[1]

Temperature[2] (° C.)	Average	Number	Range	Date
Individual A:				
27–28	80	11	20– 92	1-10-46
22–25	85	11	60– 90	1-11-46
27–28	94	4	85–105	1-11-46
28–29*	77	10	60– 87	1-12-46
19–21	141	8	133–151	1-12-46
16–17	163	16	42–450	1-14-46
17–18	180	19	83–360	1-15-46
Individual B:				
25.5	108	5	53–180	1-18-46
27 –28	111	7	88–177	1-22-46
27 –28	62	21	30– 90	1-24-46
14.5–16	226	6	200–260	1-16-46
16 –18	230	20	133–360	1-17-46

[1] Seen with Zeiss water-immersion lens D* and Spencer ocular X (except * with ocular × 6).

[2] The temperatures were roughly those of the 30–40 cc. of water in Petri dish which held the slide on which the test of folliculinid was attached.

Whether this opening of the bottom of the food sack and the departure of the food mass is ever rhythmic was not determined. But at times stained yeasts or *Euglena* pass down the esophagus in quick series without any such rhythm as described above.

The walls of the esophagus apparently are responsible for the conduction of the food down its length, as well as for its pinching-off at its mouth. In the structure of the esophagus there are many lengthwise fibrils and at times a definite lumen is seen, but no boundary between the tract and the surrounding endoplasm. The impression is that something like peristaltic contraction takes place, and Sahrhage ventured the supposition that the fibrils might be continuations of the myonemes of the funnel and might be contractile elements. In stentor, large food objects may be squeezed down the funnel by means of its peristaltic contractions, according to Dierks (1927). No rhythmic ingestion has been observed in stentor, and Gelei (1925) found in cases of cannibalism that the great ingested mass took 3 days to digest and that no other food was taken; so ingestion seems to have been deferred.

The pinching-off of food vacuoles and their transit through an esophageal tract is found in many ciliates; and, of late, evidence has accumulated to indicate that both the pinching-off and the transit are due to the contraction of flagella-like fibrils that form all, or most, of the esophageal apparatus.

It may well be that the fibrils in the walls of the esophagus of *P. amphora* may have the same contracting ability.

Flagella are essentially like cilia which, like the fibrils of heart muscle, readily take on rhythmic activities that are influenced by temperature just as are the rhythmic activities of ingestion in *P. amphora;* thus the occurrence of rhythms in ingestion supplies some support to the conception that the action is due to fine fibrils having the potency of cilia or flagella.

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THE EFFECT OF CERTAIN DEFICIENT MEDIA ON RESTING CYST FORMATION IN COLPODA DUODENARIA[1]

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THE experiments of Taylor and Strickland (1938, 1939) on *Colpoda duodenaria* have shown conclusively that this ciliate forms resting cysts when food is absent or becomes scarce in the surrounding medium. Since food in these experiments meant living bacteria (*Pseudomonas fluorescens*) in a balanced salt solution, a number of substances was actually involved. At that time, however, none of the substances composing this complete food could be tested separately. A more specific analysis of the food factor in relation to resting cyst formation, though desirable, could not be made until some of the nutrients and growth factors required by the organism were known.

The sterile nutrient medium devised by Taylor and Van Wagtendonk (1941) was a step in this direction. It consisted of a heat-stable, yeast-extract fraction and a heat-labile, bacterial-plasmoptyzate fraction, both necessary to the growth and reproduction of *Colpoda*. Fortunately, later studies (Van Wagtendonk and Taylor, 1942; Tatum, Garnjobst, and Taylor, 1942; and Garnjobst, Tatum, and Taylor, 1943) on the nutritional requirements proved that some of the substances of this complex medium could be eliminated. At present the yeast fraction can be replaced with a solution of five vitamins—thiamin, pyridoxin, calcium pantothenate, nicotinamide, and riboflavin. In contrast, the plasmoptyzate fraction to date remains relatively complex. It is chiefly protein and, in the purest form obtainable with present methods, also contains at least biotin and riboflavin, traces of the other four B vitamins mentioned, and certain unknown heat-stable substances in very small quantity which appear to be required by *Colpoda*.

Although the complex heat-labile and unknown portions of the simplified medium prevent complete analysis, experiments have been made to test singly by their presence or absence in various combinations some of the substances collectively known as the "food factor," in order to discover whether or not they affect or influence the formation of resting cysts. Briefly stated, a deficiency in any one or more of four of the vitamins required (B_1, B_6, pantothenic acid, and nicotinamide); the absence of the heat-labile factor, as in heat-treated bacterial plasmoptyzate, or the absence of the whole bacterial-plasmoptyzate fraction will lead to encystment. Under the circumstances described, the cysts formed are either rhythmic[3] (temporary) or permanent (true resting cysts).

MATERIAL AND METHODS

The vitamin–bacterial-plasmoptyzate (VBP) medium described and adopted

[1] This study was supported by a grant from the Rockefeller Foundation.

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[3] I.e., ". . . . the Colpoda will encyst but spontaneously reorganize and excyst, and this process will be repeated until, owing to depletion of the body substance, death ensues" (Strickland, 1940, p. 356).

as standard in 1943 (Garnjobst, Tatum, and Taylor) contains, per milliliter of modified Osterhout's solution,[4] 20γ each of thiamin, pyridoxin, calcium pantothenate, and nicotinamide; 5γ of riboflavin; and about 100γ of purified bacterial plasmoptyzate.[5] With a few exceptions, the test media in this investigation were one or more of the required fractions named.

For the stock cultures of *Colpoda* the standard medium was modified as follows: (1) 0.25 per cent Difco yeast extract was included to prevent deficiency over a long period of time in any of the unknown substances present in the plasmoptyzate in small and possibly variable amounts; (2) this supplement made the addition of trace elements unnecessary, and therefore these were omitted; (3) the plasmoptyzate was washed only once before resuspending it in Osterhout's balanced salt solution for the final dilution; and (4) riboflavin was reduced to 2γ/ml of medium, since it is known that an appreciable amount is already present in the plasmoptyzate prepared in this way.

The same strain of *Colpoda* used previously in the Stanford laboratories was employed. The organisms for this study were obtained in July, 1945, from resting cysts formed in the VBP medium in January, 1943. These cysts had been dried.

The *Colpoda* inoculated into test media were grown in the stock medium without the yeast supplement and were small forms recently emerged either from reproductive cysts (preferably small quadrigenics) or from permanent resting cysts formed in this medium.

[4] Supplemented with the following trace elements: (mg/liter) Bo, 0.01; Cu, 0.04; Fe, 0.02; Mn, 0.02; Mo, 0.02; Zn, 0.15.

[5] Prepared from *Pseudomonas* (*Photobacterium* Sp.), M.D. 33.2, Hopkins Marine Station, Pacific Grove, California.

True resting cysts were obtained in quantity by inoculating tubes of the modified stock medium with a great number of small, even-sized organisms taken from a stock culture. After a period of growth and multiplication and consequent clearing of the medium, many resting cysts appeared. These formed large, irregular groups on the sides of the test tube, where they remained attached during replacement of the medium with Osterhout's solution. When the cysts were 24–48 hours old, 1.0 per cent Difco yeast extract was used to induce excystment. The organisms excysting within 3 hours were washed in Osterhout's solution three or four times by centrifugation and then dispersed into the required number of Columbia watch glasses inclosed in Petri dishes. Inoculations from these dishes were made either before or after some dilution.

All media were tested for contaminants at the beginning and end of each experiment. The experiments, as usual, were made in a constant-temperature room maintained at 20° C., in which the humidity was increased from 65 to around 80 per cent saturation when drops of media were being prepared and inoculated. Differences in these and special techniques are mentioned in connection with the experiment concerned.

PRELIMINARY EXPERIMENTS

The effect of known concentrations of vitamins on encystment was observed on *Colpoda* grown in Osterhout's balanced salt solution with a quantity of *P. fluorescens* from nutrient agar. There were two reasons for returning to this method of culture (Barker and Taylor, 1931) for these experiments: (1) the ciliates could more easily be freed from all vitamins than could those grown in the new medium; and (2) the technique (Taylor and Strickland, 1938, 1939) for

obtaining numerous organisms of the same age and very nearly free from bacteria and organic solutes was well known. The results in the vitamin solution to be tested, therefore, could be compared favorably with previous findings in Osterhout's solution and also directly with a control prepared at the same time.

Approximately one-half of the organisms washed at one time were finally concentrated in the sterile vitamin solution to be tested, and the other half in sterile Osterhout's. No ill-effects were noted in any individuals as the result of the rather abrupt change from one medium to another. The volume of the drop for the encystment experiments was 1/200 ml. The number of organisms per drop was greater than two thousand, as determined by sample counts. Although the concentration of *Colpoda* varied between experiments, the differences in drops of the same experiment were slight.

The ciliates were transferred to sterilized cover slips with specially calibrated pipettes. Two separate drops containing them were put on each cover slip, and two such preparations were made for each medium. These two media were: (1) the Osterhout's solution and (2) the same solution containing one or more vitamins. At the end of the encystment period (about 3 hours) one cover slip of each set was placed under irrigation for 24–28 hours. The remaining cover slips were placed in a moist chamber for the same length of time. After the 24–28-hour period, which allows for completion of the nuclear reorganization (Taylor and Garnjobst, 1941), excystment was successfully induced in all four cyst preparations.

All the B vitamins were investigated. They were tested in the concentrations used previously (Tatum, Garnjobst, and Taylor, 1942), except the five required vitamins for *Colpoda*, which, when used separately or in combination, were in the concentrations given in the standard medium. Biotin (0.00025γ/ml) was also tested in combination with the required vitamins and separately in three concentrations—0.00025, 0.012, and 0.25γ/ml.

In all, eighteen different vitamin solutions were tested, and in all of them *Colpoda* formed numerous resting cysts. Furthermore, in the test medium, as compared with the control, no really significant difference was found (1) in the time of appearance of cysts in the drops, (2) in number of free-swimming organisms present near the end of the encystment period, or (3) in excystment time of the cysts irrigated. Therefore, it was concluded that a rather high concentration of the tested vitamins does not prevent the formation of resting cysts in this protozoan; and the absence of some of them in the absence of suitable nutrients, the bacterial plasmoptyzate, does not seem to alter the situation to any noticeable extent.

Two other preliminary experiments were made with *Colpoda* fed living *Pseudomonas*. Numerous organisms were concentrated in 0.25-ml. drops of balanced salt solution. To medium No. 1 was added 0.1 ml. plasmoptyzate, freshly prepared; to No. 2, the same amount of this plasmoptyzate autoclaved; and to No. 3, 0.1 ml. Osterhout's solution. The results of this experiment indicated no difference in time of appearance of cysts in the three media, but in No. 3 the number increased more rapidly than in Nos. 1 and 2. Both autoclaved and untreated plasmoptyzate caused a definite decrease in movement of the organisms, but in less than 30 minutes movement again became rapid. This observation is significant because rapid movements are characteristic of these ciliates in food-free media and especially during the late

precyst period. True resting cysts were formed in all three media, and excystment was successfully induced later. In order to avoid growth of bacteria, all three preparations had been irrigated as soon as most of the organisms had encysted.

Other drops of 0.25 ml. were then prepared with organisms concentrated (1) in the standard vitamin solution and (2) in Osterhout's alone. To each was added 0.1 ml. concentrated bacterial plasmoptyzate or a similarly concentrated drop of heat-treated plasmoptyzate. Although the animals promptly became less active in all drops, only in the complete medium was it noticeable later that they had taken in food and that encystment had been greatly delayed (beyond 3 hours).

Entirely reliable results could not be expected from this type of experiment. A few living bacteria were undoubtedly present, and these would sooner or later complicate the results. The remaining experiments on vitamins involving also the plasmoptyzate fraction were therefore made on *Colpoda* grown in sterile nutrient medium, using sterile techniques throughout.

RESTING CYSTS IN DEFICIENT MEDIA

From previous experience in growing *Colpoda* in the standard VBP medium it was already known that normal resting cysts appeared in a culture if the concentration of organisms became high (about 16,000/ml or more). If the number of organisms remained low, as in media with a smaller initial amount of bacterial plasmoptyzate, fewer cysts appeared. One hundred per cent resting cyst formation was never observed. Usually, a few free-swimming forms, as well, were present for a considerable time. In other cultures most of the cysts were rhythmic in nature, since their number and pattern of arrangement varied greatly from time to time.

It seemed evident from these observations that the environmental conditions of encystment are similar to those in Osterhout's solution with living bacteria as food. But which fractions, if any, are concerned in the new medium for the appearance of resting cysts? It is known from tests made earlier (Garnjobst, Tatum, and Taylor, 1943) that the concentrations of the five required vitamins selected for the standard medium are in excess of the amount needed by *Colpoda* for about 100γ plasmoptyzate per milliliter of medium. For this reason depletion of any one or more of these vitamins does not account for the appearance of resting cysts. This elimination centers interest in the plasmoptyzate fraction. It is true that the sterile nutrient medium becomes clear before cysts are formed, and the conclusion could be drawn that the gross nutrients (and/or the unknown substances) were nearly depleted. In fact, if in two tubes, containing equal amounts of medium, all fractions are the same except the plasmoptyzate, which is one-half as concentrated in one tube as in the other, resting cysts (rhythmic or permanent) will appear first in the tube containing the lower concentration, provided the same number of organisms was inoculated and sufficient oxygen was assured. The absence or near-absence of other fractions then might also lead to encystment.

One point of interest in this direction was observed in certain media deficient in one or more of the required vitamins and in plasmoptyzate without added vitamins. The animals inoculated into these media multiplied very slowly and soon tended to decrease in number, although free-swimming organisms persisted for several days. In making counts of organisms on the third or fourth day

after inoculation, small cysts were occasionally seen, and sometimes excystments which resulted in the exit of one organism from the cyst membranes. Further experiments were therefore planned to observe the sequence of events more closely in similar media.

Drops of $\frac{1}{40}$ ml. were selected for these experiments. It was not possible for technical reasons to prepare more than one test series per day; therefore, each of the four series contained freshly prepared plasmoptyzate. To provide a basis for comparison of the deficient media, in the absence of completely standardized plasmoptyzate, each series, beginning with the second, contained one or two test media of the previous series. With a finely calibrated pipette, $\frac{1}{40}$-ml. drops were distributed (in triplicate for each medium) to concavities of agglutination slides with twelve concavities. Each multidepression slide was inclosed in a Petri dish with added droplets of water inside to prevent evaporation. At the end of the experiment, three or four drops of the experimental media, chosen at random, were removed to test for contaminants. The *Colpoda* inoculated had recently emerged from resting cysts. The inoculum was an extremely small droplet of water containing one individual (or more in other experiments). Unless the organisms were unusually small—and these were avoided—there were no early casualties in the drops containing one organism; and no drop was inoculated a second time.

The behavior of *Colpoda* at any time and at high magnification was observed in Carrel flasks made of very thin, clear glass. Before these flasks were sterilized, a washed disk of filter paper with a very large hole cut out of the center was slipped into each and flattened to the bottom. This narrow border of paper was dampened with water some time before the drops of media containing the ciliates were placed on the upper inside surface with a finely drawn, curved pipette.

The first series of deficient media tested was prepared by omitting the four vitamins, one at a time, from the standard medium with appropriate controls. Riboflavin was not considered at this time because the plasmoptyzate retains enough of it throughout the washing process to permit growth (Garnjobst, Tatum, and Taylor, 1943). The results therefore would not be clear unless some other method was used to remove the remaining riboflavin. The media of the second and third series were made deficient in two of these vitamins. The fourth series contained only one vitamin in each medium tested, three of those required having been omitted.

The results of these experiments are given in Table 1. Although several sets of figures were obtained for each series, only one representative set of each was selected for this paper. It will be seen that, in all the deficient media, *Colpoda* formed resting cysts after a period of extremely slow growth and division. On the other hand, considerable growth and division occurred before such cysts appeared in the complete medium of each experiment. In all drops containing incomplete media some plasmoptyzate remained uneaten at the end of the experiment. This material was easily recognizable in the concavities of the depression slide because it settled down upon standing. The number of organisms produced seemed to decrease as the number of vitamins omitted was increased, although extremely accurate measurements using the same bacterial plasmoptyzate throughout would be necessary to prove this point precisely. The number of organisms in each of the three drops of the same medium, however, was reasonably uniform; in fact, it was more

uniform than is usual, since the organisms tended to divide digenically rather than quadrigenically. The difference in number of individuals produced in the test media as compared with the control (the complete medium) was always great.

These figures are fully supported by the results obtained in previous experiments (Garnjobst, Tatum, and Taylor, 1943) of a similar kind. The difference in number of individuals produced in the deficient media as compared with the complete medium was even greater when 1-ml. amounts were used. This difference probably results from the inability of the organisms to ingest some of the plasmoptyzate held in the surface of the drop.

The information gained in the present experiments concerned not so much the amount of growth possible in $\frac{1}{40}$-ml. drops of the various media as the time of appearance and the nature of the resting cysts. The pre-encystment period in the deficient media varied from 2 to 6 days. It also varied in the same medium when experiments were repeated, though con-

TABLE 1

RESTING CYST FORMATION IN VITAMIN-DEFICIENT MEDIA

Amount of medium in drop—$\frac{1}{40}$ ml., inoculum—1 *Colpoda*. "C"—Control.

Required Vitamins Omitted*	No. of Organisms Present in Drop (Av. of 3 Drops)						
	1st Day	2d Day	3d Day	4th Day	5th Day	6th Day	7th Day
Series 1:							
1. B_1	2	3	6	7	Discontinued		
2. B_6	4	4	11	16†	Discontinued		
3. Calcium pantothenate	3	6	12	20†	Discontinued		
4. Nicotinamide	4	7	12†	12	Discontinued		
C. None	5	26	72	165	166† (144–92)		
C. All four vitamins	2	6	6†	7	6	Discontinued	
Series 2:							
1. B_1	1	4	4	5	6	13†	9
2. B_2	1	7	7	7	7†	8	4
3. Calcium pantothenate	1	2	3	4†	4	4	3
4. Nicotinamide	1	4	4	6	7	14†	9
C. None	2	6	12	32	61	64	112† (86–140)
C. All four vitamins	1	1	2	2	1	0	0
Series 3:							
1. B_1	1	3	4	6	6	12†	7
2. B_6	1	6	6	7†	5	5	4
3. Calcium pantothenate	1	2	3	3	4†	5	5
4. Nicotinamide	1	2	2	3	5†	5	3
C. None	1	9	13	34	68	70	128 (102–42)
C. All four vitamins	1	1	2	2†	1	1	0
Series 4:							
1. All except B_1	1	1	1	1	2†	1	0
2. All except B_6	1	2	2	2†	2	2	1
3. All except calcium pantothenate	1	1	2	4†	2	2	3
4. All except nicotinamide	1	1	2	2	2†	3	2
C. None	1	4	8	9	16	48	122 (108–36)
C. All four vitamins	1	1	1	2	3†	2	2

* Riboflavin is also required by *C. duodenaria* but was not considered in this experiment.

† Indicates the day that resting cysts (rhythmic) were formed.

sistency could hardly be expected with unavoidable slight differences in the bacterial plasmoptyzate. All the cysts were of the rhythmic type, except in some drops containing the complete medium. A large number of reproductive cysts excysting at about the same time favored the appearance of true resting cysts in these controls.

The series of media deficient in only one vitamin each was further tested with more than one individual per drop. In Experiment I the drops contained 10–25 organisms. Usually very slow growth and a few digenic cysts appeared before rhythmic cysts were formed (18–48 hours), but no quadrigenic division cysts were seen except in the drops of the complete medium. In other series of this same experiment, possibly because in some media the washing removed more or all of the vitamins present in the plasmoptyzate or a slight difference resulted in the concentration of this fraction, rhythmic cysts appeared within 9–12 hours. No reproduction occurred during this time, although it was noticeable that the organisms had grown slightly larger. In all drops of the complete medium the organisms continued to grow for a much longer time.

It was not considered a part of this investigation to determine the number of ciliates which must be present in each of the deficient media in order to produce any true resting cysts. That such cysts are dependent upon the number of free-swimming organisms present in the drop was evident in Experiment II, with the same series of deficient media as in Experiment I but containing more than 100 individuals per drop. In these drops, encystment began after a free-swimming period of only 4 hours, whereas the animals in the drops of the complete medium continued to grow larger after this time. Some of the cysts in the incomplete media proved to be of the permanent type.

The number of true resting cysts per drop was determined for two identical series of media deficient in one vitamin each, except that the same plasmoptyzate was not used. The results are given in Table 2.

The preliminary experiments described earlier in this paper were, in part, repeated with organisms grown in sterile nutrient medium. The vitamins of the standard medium and various combinations of them were the only ones tested, although biotin was also added to this list. Again, resting cysts were readily formed in the vitamin solution, as well as in Osterhout's.

The same results were obtained when numerous small organisms were concentrated in 0.25-ml. drops of the standard vitamin solution to which 0.1 ml. concentrated plasmoptyzate was added or the same amount of plasmoptyzate heat-treated. In the first medium the ciliates became opaque with food, and reproductive cysts were eventually formed. In the second medium the organisms resumed active movement after a period of semi-quiescence lasting about 30 minutes. Encystment without division followed after 1 hour and 15 minutes. In all repetitions of this experiment, true resting cysts were formed in the deficient, as well as in the complete, medium, if the number of organisms per drop was high. This number was always greater than would have been necessary if the medium had been Osterhout's solution alone.

EXCYSTMENT EXPERIMENTS

During this study it was discovered that the standard VBP medium appears to contain very little, if any, of the factors causing excystment. Six test tubes containing 5-ml. amounts of this medium were inoculated with a large num-

ber of small *Colpoda*. After about 48 hours there happened to be present in one of these tubes numerous large clusters of quadrigenic division cysts; within 2 hours a great many small organisms had conveniently collected in the upper half of the medium. With a special pipette, 3 drops (volume, $\frac{1}{400}$ ml.) of these organisms were put in a row on the inside glass surface of ten small test tubes previously plugged with cotton and sterilized. As soon as resting cysts had formed and very few free-swimming organisms remained in the drops, 1.5 ml. of sterile Osterhout's solution were pipetted into the tubes. The cysts, of course, remained attached to the glass in three rounded areas, readily visible under low magnification. Each area contained more than five hundred cysts. The tubes were left standing for 24 hours, after which the cysts were washed several times by removal of the medium and readdition of fresh Osterhout's solution. When no free-swimming organisms were finally present and the cysts were about 40 hours old, the preparations were ready for the excystment experiments.

The following media were added to the tubes containing cysts, with two tubes for each medium: (1) the standard VBP medium; (2) the same medium containing bacterial plasmoptyzate twice as concentrated as in No. 1; (3) 1.0 per cent Difco yeast extract; and (4) Osterhout's balanced salt solution. In medium 3, excystment began in 135 minutes and was completed within 4 hours. No excystments took place in any of the other tubes, although the tubes were left standing for 48 hours, and one series for 4 days. After 48 hours, 1.0 per cent yeast extract was added to one set of the remaining tubes. Within 6 hours most of the Colpoda had excysted. The three to six cysts remaining in each tube were placed under higher magnification. The organisms were rotating inside the membranes but were experiencing difficulty in making their exit.

TABLE 2

TRUE RESTING CYST FORMATION IN $\frac{1}{40}$ ML. OF DEFICIENT MEDIA

Medium Deficient in—	Total No. of Colpoda Present in Drop	Total No. of Free-swimming Colpoda Removed	Total No. of True Resting Cysts
Series 1:			
Thiamin	384	31	353
Pyridoxin	373	59	314
Calcium pantothenate	392	38	354
Nicotinamide	368	21	347
All four vitamins	356	26	330
Series 2:			
Thiamin	196	190	6
Pyridoxin	189	184	5
Calcium pantothenate	192	185	7
Nicotinamide	190	185	5
All four vitamins	128	125	3

DISCUSSION

It has been shown in the foregoing account of experiments made that all fractions of the standard vitamin–bacterial-plasmoptyzate medium are required to

maintain the fully differentiated, free-swimming state. If any one of them is omitted, *C. duodenaria* will form resting cysts.

A period of slow growth and reproduction may or may not precede the formation of these cysts. The varying length of this period probably means (1) either that the medium was not completely deficient in the fraction omitted, (2) that the organism contained a limited supply of it, or (3) both. The results obtained in the bacterial plasmoptyzate alone (see Table 1) indicate that in most cases the fraction was not completely deficient in the vitamins required. If incomplete deficiency existed in any medium, it would be possible to decrease the period of growth by introducing more than one organism into the drop, and this actually proved to be true. No reproduction then occurred. Regarding 2 above, it will be recalled that the *Colpoda* inoculated had recently emerged from permanent resting cysts. This means that these organisms were small and devoid of food, and probably of food reserves; but it also means that they spent some time in the excystment medium, 1.0 per cent yeast extract. Although it seems unlikely that food taken in during 2–3 hours in this extract would be sufficient to produce a digenic division cyst (Taylor and Strickland, 1938), it might give energy for a longer free-swimming period. If so, at least some of it would be used up in the washing period.

True resting cysts formed in the deficient media appeared to be entirely normal, as shown in behavior during induced excystment and in general structure of the excysted organism. Individuals forming temporary resting cysts were able to excyst and re-encyst several times; but in some media after the second encystment the free-swimming ciliate showed signs of degeneracy, such as peculiarity in movement and coarse granular cytoplasm. No detailed study of these differences was made. The organisms finally died either inside the cyst membranes or during the free-swimming state.

Rhythmic cysts are also formed by *C. duodenaria* in 1.0 per cent Difco yeast extract (Taylor and Strickland, 1939). This medium was later found to be deficient (low) in thiamin and pantothenic acid (Garnjobst, Tatum, and Taylor, 1943), and, of course, it also lacks the heat-labile factor.

Colpoda, in nature, might encounter environments resembling the deficient media described in this paper. Some bacteria suitable as far as the gross nutrients are concerned might not produce all the vitamins necessary. Or the bacteria available might be deficient in some necessary protein constituent. Thus resting cysts might well be formed in the laboratory or in nature, in the presence of bacteria unsuitable as a complete food; and if the number of these ciliates was great enough, permanent cysts would undoubtedly be formed also.

The conclusions of Taylor and Strickland (1938) that encystment follows the reduction in the amount of the available food supply in the medium was based upon experiments made with a *complete* food—living *Pseudomonas*. The present study has shown that the food supply also can be reduced by causing a deficiency in any one or more of the known essential fractions of the complete medium containing no living bacteria. In other words, certain specific deficiencies in food, as well as an insufficient quantity of food, induce the formation of resting cysts in *Colpoda*.

The important question regarding the factor responsible for the production of

true resting cysts remains unsolved. According to Strickland (1940), the encystment-promoting factor is not identical with the factor inducing the change from cyst to true resting cyst. He has found that the potency only of the latter varies with the volume of the drop. Apparently as a consequence of the difference, the macronucleus of the true resting cyst undergoes a distinct reorganization. It is hoped that further studies with sterile techniques will give additional facts regarding the formation of this greatly condensed and dedifferentiated stage.

SUMMARY

Specific deficiencies in the standard vitamin–bacterial-plasmoptyzate medium were produced by omitting one or more of four of the vitamins (thiamin, pyridoxin, calcium pantothenate, and nicotinamide) or the bacterial-plasmoptyzate fraction or by substituting heat-treated for untreated bacterial plasmoptyzate.

Colpoda duodenaria inoculated into these media formed resting cysts. Whether rhythmic cysts or true resting cysts were formed depended upon the concentration of the organisms in a given drop. In general, the greater the number of free-swimming organisms, the greater the number of true resting cysts were formed.

The standard vitamin–bacterial-plasmoptyzate medium contains very little, if any, of the factors which induce excystment in *Colpoda*.

ACKNOWLEDGMENT.—The experiments described in this paper were planned with Professor C. V. Taylor. The results were known before his untimely death on February 22, 1946; but he was unable to review the written account. The author is grateful for the help and inspiration of a former teacher and long-time associate but assumes full responsibility for the presentation of the material reported here.

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SOME INTERRELATIONS OF DRUG AND ION ACTIONS IN THE ARTIFICIAL ACTIVATION OF MARINE EGGS

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THE experiments to be reported here were begun with the aim of examining possible actions of acetylcholine and related compounds on the eggs of marine animals. It was observed early in the course of the work that acetylcholine or physostigmine in small concentrations (1 part in 10,000) served to inhibit activation of marine eggs by any of several agents (Scheer, 1945). Further examination of this effect showed it to be related especially to the action of calcium ion and, to a lesser degree, to the action of other ions in isotonic solutions.

It has been necessary to interrupt this work in mid-course, and some of the results presented here are preliminary in nature. However, it is believed that they are of sufficient importance to justify a presentation at this time. We hope to be able to continue the work at a later date.

Recent physiological and biochemical literature has included many studies pointing to interrelations of calcium ion and adenosine triphosphate, on the one hand, and acetylcholine and related drugs, on the other, in the excitation of muscle and nerve. The distribution of choline esterase in the electric organs of certain fishes and in the giant axons of the squid suggests that acetylcholine is closely concerned with the electrical changes in these tissues (Fulton and Nachmansohn, 1943). Potassium ion is likewise considered as important in electrical changes in nerve and muscle (Bishop, 1932; Cowan, 1934; Fenn, 1936; Wills and Fenn, 1938; Young, 1938; Tipton, 1938; Brown and McIntosh, 1939; Heppel, 1940; Fenn, 1940). Moreover, potassium ion causes formation and liberation of acetylcholine in nerve and other tissues (Beznak, 1934; Feldberg and Guimarais, 1935; Brown and Feldberg, 1935; Mann *et al.*, 1939; Welsh and Hyde, 1944; Feldberg, 1944; Feldberg and Mann, 1944). The mechanism of this action appears to be related to the probable role of adenosine triphosphate in the synthesis of acetylcholine (Welsh and Hyde, 1944; Feldberg, 1944; Feldberg and Mann, 1944). Either potassium ion (Bethe and Franke, 1925) or acetylcholine will produce typical contractions in muscle, and the two effects are related, though not identical (Wachholder and Mathias, 1933; Cicardo, 1939).

The interrelations with calcium ion are less clear. It is usually found that calcium and potassium ions are antagonistic in their effects on biological processes. Heilbrunn (1937, 1940) has stressed the importance of calcium in excitation. Calcium ion activates the adenosine triphosphatase of muscle, which latter is closely associated with myosin (Engelhardt and Ljubimowa, 1939; Bailey, 1942; Needham *et al.*, 1941, 1942; Price and Cori, 1946). Indeed, Bailey (1942) has stated that "the essential feature of excitation and contraction is the liberation of Ca^{++} in the neighborhood of myosin." This statement has not yet been seriously challenged. Potassium

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ion has little effect on this reaction. A relation of acetylcholine to the picture has been suggested by DuBois and Potter (1943), who, in a short note, reported that, in the absence of calcium ion, the adenosine triphosphatase of muscle is activated by small amounts of acetylcholine. This has not been confirmed as yet.

Marine eggs have long been favorite subjects for the study of ion actions. Activation has been observed in unfertilized eggs as the result of treatment with isotonic sodium chloride (*Asterias* [Lillie, 1910]), and calcium chloride solutions (*Thalassema* [Hobson, 1928]; *Cumingia*, *Arbacia* [Hollingsworth, 1941]). The addition of potassium chloride to sea water has been found to activate the eggs of *Nereis* (Wilbur, 1941), and *Amphitrite* eggs have been activated by addition of calcium chloride to sea water (Fischer, 1902). Lillie (1910, 1911, 1913, 1916, 1921, 1932) has attributed the action of isotonic salts to their effect in increasing permeability. The general field of artificial activation has recently been reviewed by Tyler (1941*a*). He has examined the calcium theory of Heilbrunn in some detail.

Mirsky (1936*a*) has reported the existence in unfertilized eggs of a protein which is soluble in 1 M potassium chloride at pH 7, but which becomes insoluble under these conditions after fertilization. Myosin in muscle undergoes a similar change in rigor (Mirsky, 1936*b*). Heilbrunn and Wilbur (1937) and Wilbur (1939, 1941) have pointed out that activation of *Nereis* eggs by ultraviolet light is probably associated with liberation of calcium ion in the cortex. Wilbur (1939) has shown that activation by ultraviolet is inhibited by magnesium ion, but that this inhibition is overcome by calcium ion. Calcium and magnesium ions are antagonistic in their action on adenosine triphosphatase from muscle or from electric organs (Bailey, 1939; Greville and Lehmann, 1943). These results do not yet form a coherent picture, but they indicate that liberation of calcium from an intracellular compound, activation of adenosine triphosphatase, movement of potassium across the cell membrane, and formation or liberation of acetylcholine may all be important in excitations generally.

MATERIAL AND METHODS

For most of the experiments reported here, the eggs of the echiuroid worm, *Urechis caupo* Fisher and MacGinitie, were used. The worms were collected near the Kerckhoff Marine Laboratory and kept in sand and running sea water until used. The eggs were removed from a single gonopore by pipette and transferred to finger bowls containing about 50 cc. of freshly filtered sea water. The eggs were ordinarily used within 30 minutes after removal from the animal.

Breakdown of the membrane surrounding the germinal vesicle was used as the criterion for activation in this work. Membrane formation and cleavage, though usually present, were not so dependable, and polar-body formation was less easily observed.

In most of the experiments the eggs were removed from the finger bowls, with a minimum amount of sea water, to Syracuse dishes containing about 10 cc. of the experimental solution. As soon as the eggs had settled, the solution was drawn off and replaced with fresh solution to remove traces of sea water introduced with the eggs. At intervals, 200–500 eggs in each dish were counted, and the number of eggs activated (germinal vesicle gone) and dead or cytolyzed was recorded. Records were also kept of membrane elevation and polar-body formation; but these, in general, paralleled

the disappearance of the germinal vesicle. In Figures 1–8 the open circles connected by solid lines indicate the percentage of eggs activated at any particular time after transfer to the experimental solution, while the dots connected by broken lines indicate the percentage of eggs cytolyzed. Evident darkening or vacuolation of the cytoplasm or rupture of the cell membrane was considered evidence of death or cytolysis.

It has been brought to our attention that 0.367 M solutions of calcium chloride and magnesium chloride, theoretically isotonic with sea water, are not actually so in the case of marine eggs because of the fact that the egg membrane is not a perfect semipermeable membrane. It is, accordingly, possible that some of the results obtained with solutions in which these salts were present in quantity should be attributed to osmotic factors.

TABLE 1

FORMULAE FOR SOLUTIONS USED IN MAKING ARTIFICIAL SEA WATER

Ingredients	Grams per Liter	Concentration (Mols/Liter)	Volume of Solution To Make 1 Liter of Artificial Sea Water (Cc.)
1. *Sodium sea water:*		0.55 (Na^+)	816
NaCl	28.1	.....	.....
Na_2SO_4	4.68	.....	.....
$NaHCO_3$	0.229	.....	.....
NaBr	0.100	.....	.....
H_3BO_3	0.013	.....	.....
NaOH to bring pH to 8.0, if necessary	.....	.....	.....
2. *Magnesium chloride:*			
$MgCl_2 \cdot 6\,H_2O$	67.6	0.367	139
3. *Calcium chloride:*			
$CaCl_2 \cdot 2\,H_2O$	49.0	0.367	26
4. *Potassium chloride:*			
KCl	41.1	0.55	17

ACTIVATION BY ISOTONIC SOLUTIONS OF THE SALTS OF SEA WATER

The solutions used are listed in Table 1. The sodium sea water was prepared to have the same anion concentration and pH as sea water, but with sodium as the only cation. In the early work, 0.367 M calcium chloride was used. In some instances, treatment with calcium chloride for 20–30 minutes, followed by transfer to sea water, was effective in activating eggs. More frequently, however, this treatment failed to produce activation. Activation was also observed in a calcium-free sea water, prepared by adding to the amounts of isotonic potassium chloride and magnesium chloride indicated in Table 1 enough sodium sea water to make 1 liter. Such activation usually appeared only after 1 hour or more.

It was subsequently found that sodium sea water alone was a very effective activating agent, and it seemed desirable to observe the effects of other ions on activation in sodium sea water.

SODIUM SEA WATER

The action of sodium sea water on *Urechis* eggs is depicted in Figure 1. In a typical experiment, eggs were removed from the animal at 9:50 A.M. The diameter of these eggs in sea water was 44–47 units—average, 45.6. They were transferred at 10:00 to sodium sea water. The diameter was again measured and was found to be 45.3 units, indicating essential isotonicity. Ten minutes later, the indentation characteristic of the unfertilized egg had disappeared, and the egg had assumed a spherical shape, without change in diameter. By 10:30, the indentation had returned. At 10:50, membrane formation was noted. At 11:00, 5 per cent of the eggs had lost the germinal vesicle and were showing signs of cytolysis. The cytoplasm had become dark, and small vacuoles had appeared at the surface. At 11:30, 97 per cent of the eggs had lost the germinal vesicle, and 92 per cent were partly or wholly

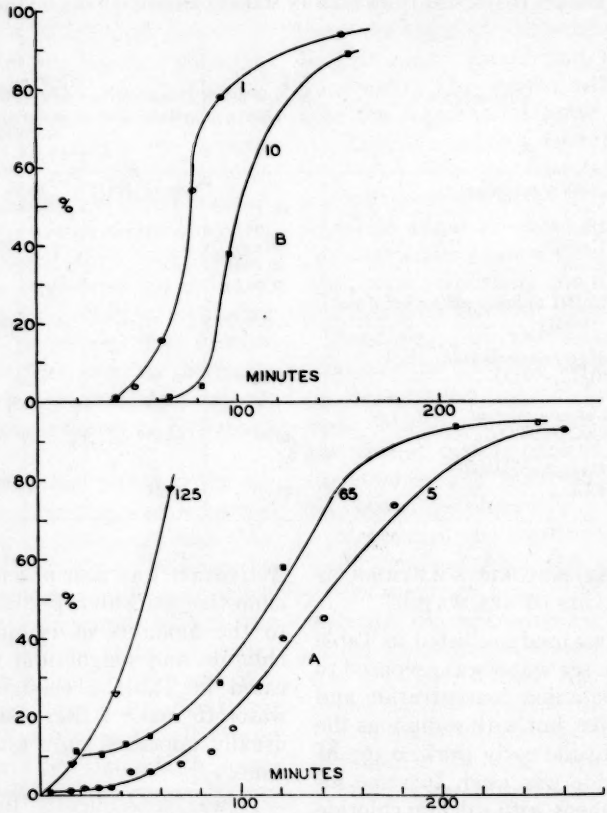

FIG. 1.—Activation of *Urechis* eggs in sodium sea water. *Ordinate*, percentage of eggs activated; *abscissa*, time in minutes. *A*, Effect of aging: The eggs were allowed to stand in sea water for 5, 65, and 125 minutes after removal from the animal, before transfer to sodium sea water. *B*, Effect of egg concentration: The dish from which the lower curve was derived contained about ten times as many eggs in the same volume of water as were present in the other dish.

cytolyzed. No change in diameter occurred until cytolysis was well under way. Evidently, cytolysis did not result from any difference in osmotic concentration. Presumably, changes in the ionic composition of the cytoplasm were responsible for the disintegration of the eggs.

Eggs were also removed from the experimental dish to sea water at intervals. Only a very small amount of activation was noted in eggs removed before 10:50, but 31 per cent of the eggs removed at 10:50 were activated, only 4 per cent being cytolyzed. These eggs had normal fertilization membranes, and several showed regular cleavage, while many formed polar bodies. This indicates that sodium sea water produced true activation, if transfer to sea water was made before cytolysis began.

The same sequence of changes—rounding-out of the egg, membrane elevation, and cytolysis—was observed in nearly all the experiments to be reported here. The time relations, however, varied considerably. Different samples of eggs, even from different gonopores of the same animal, were activated at different rates. In any particular sample the rate of activation was influenced by the time which elapsed after removal from the animal and by the concentration of eggs in the dish. Figure 1, *A*, shows the effect of "aging" in sea water on rate of activation. Eggs were removed from the animal to sea water and then transferred to sodium sea water after 5, 65, and 125 minutes. The curves indicate the percentage of the eggs which had lost the germinal vesicle at various times after transfer to sodium sea water.

Figure 1, *B*, shows the effects of crowding. One drop of a dense suspension of eggs was added to one dish, and one drop of a less dense suspension was added to a second; a subsequent count showed that the first dish (*lower curve*) contained approximately ten times as many eggs as the second (*upper curve*) in the same volume. The effect of crowding might have resulted from liberation of substances (CO_2, calcium or other ions, for example) which would retard activation. When eggs were placed in sodium sea water and repeatedly washed with sodium sea water by removing the solution and replacing with fresh solution at intervals of about 2 minutes, cytolysis appeared very soon, without preliminary breakdown of the germinal vesicle.

SODIUM PLUS POTASSIUM

The addition of isotonic potassium chloride (0.55 M) to sodium sea water in amount sufficient to bring the K^+ concentration to approximate equivalence with that prevailing in sea water[2] served to delay only slightly the changes described above and did not alter their nature. When the potassium concentration exceeded that of sea water, a distinct retardation was evident (Fig. 2). With concentrations of potassium ten to forty times that of sea water or with pure isotonic potassium chloride the rate of activation approached or exceeded the rate in sodium sea water; but cytolytic changes were delayed, so that essentially normal activation occurred in most instances. When cytolysis began in these solutions, it involved breakdown of the cell membrane rather than the cytoplasmic darkening, characteristic of sodium sea water and of mixtures with lower potassium concentrations.

It would appear, then, that potassium in high concentrations had an effect on activation which is independent of any effect on cytolysis. This view was confirmed by an experiment which, unfortunately, there has been no opportunity

[2] Such a mixture will be referred to for brevity as Na+ K.

to repeat. Eggs were washed with a mixture of sodium sea water and isotonic potassium chloride with a potassium concentration ten times that of sea water (Na + 10 K). This, like similar washing with sodium sea water, led to rapid cytolysis before disintegration of the germinal vesicle. Eggs were then treated with the washings from this experiment. The rate of activation was much higher than in fresh Na + 10 K; but cytolysis was very much delayed, as in Na + 10 K (Fig. 2). It will be noted later that a similar rapid activation with Na + 10 K occurred if calcium ion was added to this mixture.

SODIUM PLUS MAGNESIUM

The most striking effects of magnesium ion were (1) its marked retardation of activation at any concentration above one-twentieth that of sea water and (2) the formation of irregular membranes, without other evidence of activation, in high concentrations (Na + 10 Mg, 0.367 M $MgCl_2$). The latter effect might have been caused by osmotic factors, as noted earlier. In general, the rate of activation and cytolysis decreased as magnesium concentration increased.

SODIUM PLUS CALCIUM

The addition of calcium chloride to sodium sea water led to most interesting

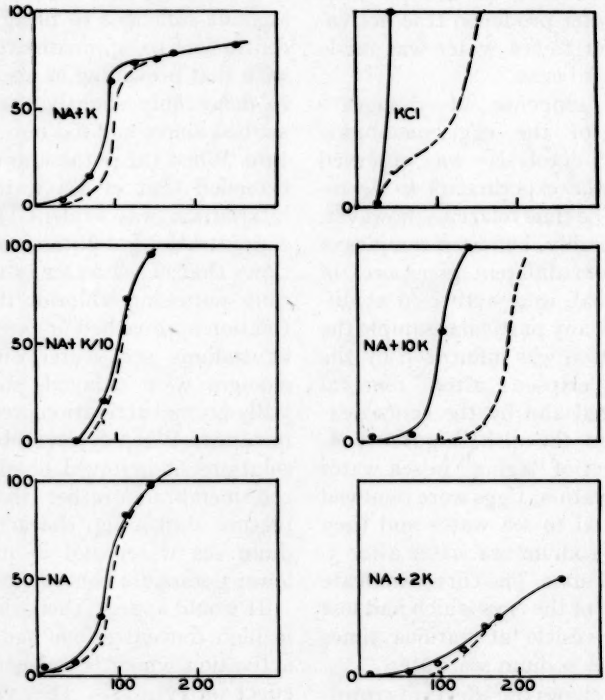

FIG. 2.—Activation of *Urechis* eggs in sodium sea water, in sodium sea water with additions of isotonic potassium chloride solutions, and in isotonic potassium chloride. Na + K represents a solution containing Na^+ and K^+ as the only cations, with K^+ in the concentration prevailing in sea water; in Na + 10 K the K^+ concentration is ten times that of sea water. Ordinate and abscissa as in Fig. 1. Solid line indicates activation; broken line indicates cytolysis.

results. In some experiments, increasing calcium concentrations led to decreased rates of activation, with a minimum at or above calcium-ion concentration of sea water. Further increase of calcium-ion concentration, however, led to an increased rate of activation, with a decrease in rate of cytolysis, somewhat like that noted earlier in the case of potassium (Fig. 3). In 0.367 M calcium chloride, cytolysis began before any disintegration of germinal vesicles was evident. However, after about 2 hours, those eggs which had not yet cytolyzed cleaved, with the germinal vesicle intact. The germinal vesicle remained in one blastomere. These eggs also cytolyzed in a short time. Again, osmotic factors were not excluded with high calcium chloride concentrations.

In other experiments the addition of small amounts of calcium chloride to sodium sea water led to very rapid activation. After a certain percentage of eggs had been activated, further activation ceased. In fact, some eggs which had earlier been regarded as activated regained the germinal vesicle. After a pause of 20–50 minutes, activation was resumed and continued until all the eggs were activated (Fig. 4, *B*, *D*). Cytolysis did not at once follow the activation of the first eggs but began only after an interval. The time at which cytolysis began, in eggs activated earlier, coincided roughly with the end of the pause in activation and with the beginning of activation and cytolysis in control eggs in sodium sea water (Fig. 4, *B*, *D*).

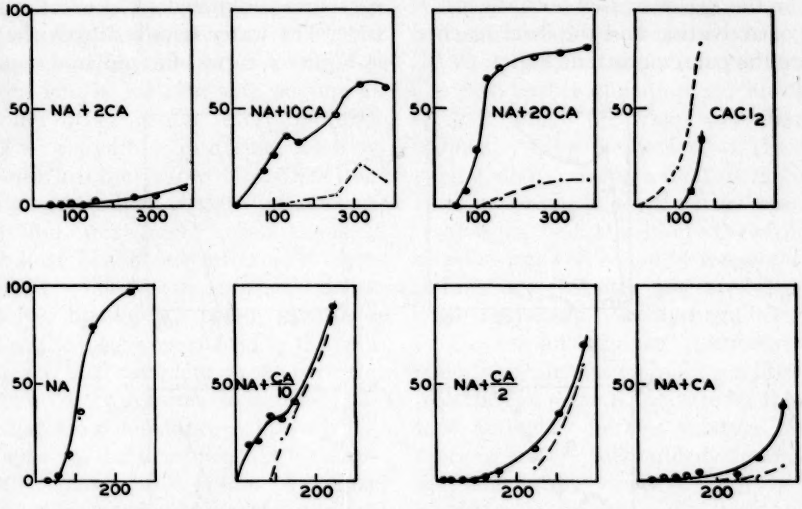

FIG. 3.—Activation of *Urechis* eggs in mixtures of sodium sea water with 0.367 M calcium chloride. Symbols as in Fig. 2.

There was apparently some seasonal factor involved in this "calcium effect." It was first observed in February and again on several occasions in March. In April it was less evident and could not be produced at all during July and August. There was some indication that this seasonal change was correlated with a decrease in the rate of activation in sodium sea water, but the data were not

adequate to establish such a correlation with certainty.

There is considerable evidence that a substance was liberated from eggs in sodium-calcium mixtures which inhibited the initial rapid activation. The manifestation of the calcium effect was markedly influenced by egg concentration; with higher egg concentration the initial phase of rapid activation was reduced or eliminated. Changing the solution on the eggs increased both the initial rate of activation and the level reached before the pause occurred (Fig. 4, *C*, *F*). Figure 4 compares the situation of two batches of eggs; the first batch was divided between sodium sea water (*A*), sodium sea water with added calcium (*B*), and sodium plus calcium, changed at 6-minute intervals after the eggs were introduced (*C*). The early rapid phase of activation appeared in many more eggs before the pause began, when the water was changed. Cytolysis was not affected, and the activation of 100 per cent of the eggs was completed at about the same time. The water removed from the eggs in Figure 4, *C*, was filtered, and a sample

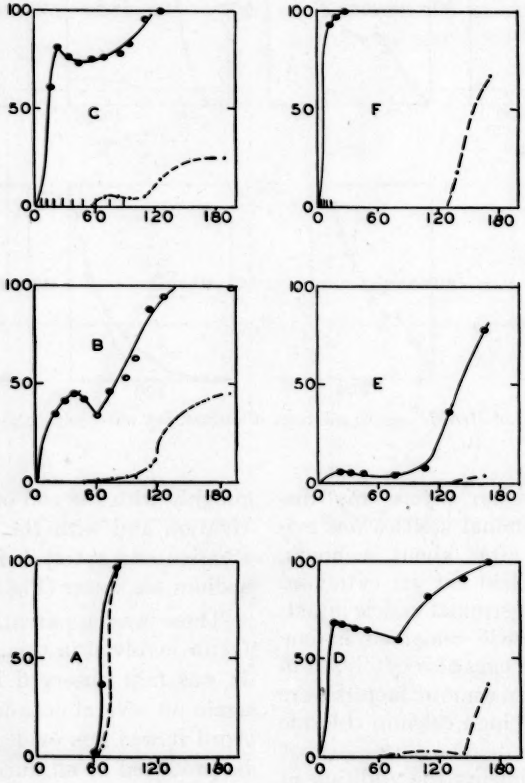

FIG. 4.—Activation of *Urechis* eggs in sodium-calcium mixtures, and the effect of repeated changes of solution. *A*, sodium sea water; *B*, sodium sea water with calcium chloride (Na + Ca); *C*, same solution as B, but changed at intervals as indicated by the marks along the abscissa; *D*, different eggs in sodium + calcium; *E*, same eggs as B, but in solution removed from eggs in C above; *F*, same eggs as D, but solution changed at intervals as indicated. Symbols as in Fig. 2.

from the second batch of eggs was placed in it (4, *E*). Activation appeared in only a few eggs during the first 2 hours, while 60 per cent of other eggs from the same batch, in fresh sodium-calcium mixture, were activated within 15 minutes (4, *D*). A third sample of the same eggs was placed in fresh sodium-calcium mixture, and the solution was changed at intervals of about 3 minutes. The eggs in this dish were all activated within 30 minutes, and over 90 per cent were activated within 15 minutes (4, *F*).

It appears from these experiments that calcium ion, in solution with sodium as the only other cation, exerts a direct effect on activation; in normal fertilization and in other types of artificial activation (distilled water, ultraviolet, other ion mixtures), germinal-vesicle breakdown begins only after 40 minutes or more, while in the sodium-calcium mixtures, breakdown began almost as soon as the eggs were placed in the solution and was complete in many cases within 10 or 15 minutes. It is also apparent that some substance is given off by the eggs in these solutions which specifically inhibits this action of calcium ion. Finally, we should mention that the eggs are not always susceptible to this action—as, for example, in the summer, when it could not be observed.

MIXTURE OF SODIUM SEA WATER WITH TWO CATION CHLORIDES

In general, the addition of two cation chlorides to sodium sea water resulted in slower activation than either one alone. In particular, in solutions approaching the composition of sea water (Na + K + Ca, Na + Mg + K), activation was greatly delayed or did not occur at all. Cytolysis was also delayed, and activation was not immediately followed by cytolysis.

Particular interest attaches to mixtures of sodium, potassium, and calcium, in view of the results described earlier. The addition of small amounts of potassium to a mixture of sodium and calcium, or of calcium to sodium and potassium, greatly retarded activation (Figs. 5, 6). With large amounts of calcium, or especially with large amounts of potassium (eight to ten times sea-water concentration), however, activation was delayed to a much smaller degree. Indeed, in Na + Ca + 8 K, activation was almost instantaneous in some cases (Fig. 6). Such instantaneous activation was observed earlier in sodium-calcium mixtures in which the water was changed frequently after immersion of the eggs (Fig. 4, *C*, *F*), and in a mixture of Na + 10 K in which eggs had earlier been activated. In the first instance the rapid activation was attributed to removal of an inhibiting substance liberated by the eggs. This could possibly have been K^+. In the second instance, it appears possible that calcium ion had been liberated into the Na + 10 K mixture by the eggs first activated in this mixture. When more eggs were then added, the mixture was comparable to Na + Ca + 10 K in its action.

ACTIVATION BY OTHER AGENTS

In an earlier paper (Scheer, 1945), the results of experiments involving activation with hypotonic sea water and with ultraviolet light have been reported. The experiments with hypotonic sea water have not been extended here. Tyler (1932) has studied this method of activation in some detail.

The use of ultraviolet light for activation is well known for other eggs but has not been described for *Urechis*. The lamp used has been described by Spikes (1944). Eggs were placed in finger bowls or Syracuse dishes at a distance of about 20 cm. from the lamp and irradiated for

short periods, usually 1–2 minutes. Activation was rapid and was not usually accompanied by cytolysis. The eggs formed regular membranes, lost the germinal vesicle, and formed polar bodies, as in normal fertilized eggs. Cleavage was observed in many, but by no means all, eggs. Tyler (1932) has pointed out that cleavage is infrequent in artificially activated eggs of *Urechis*, unless polar-body formation is suppressed.

The effects of crowding, noted earlier in experiments with isotonic salt solutions, were also apparent when the eggs were activated with ultraviolet. Eggs from the same lot were distributed in two dishes so that one dish contained approximately thirty times as many as the other, in equal volumes. The eggs were then irradiated for 1 minute. In the dish with the larger amount of eggs, only 22 per cent were activated, while in the dish with the smaller amount, 86 per cent were activated.

The possibility was investigated that, in this case as in those reported earlier, a substance liberated from the eggs was responsible for this difference. Eggs were placed in sea water and irradiated. The

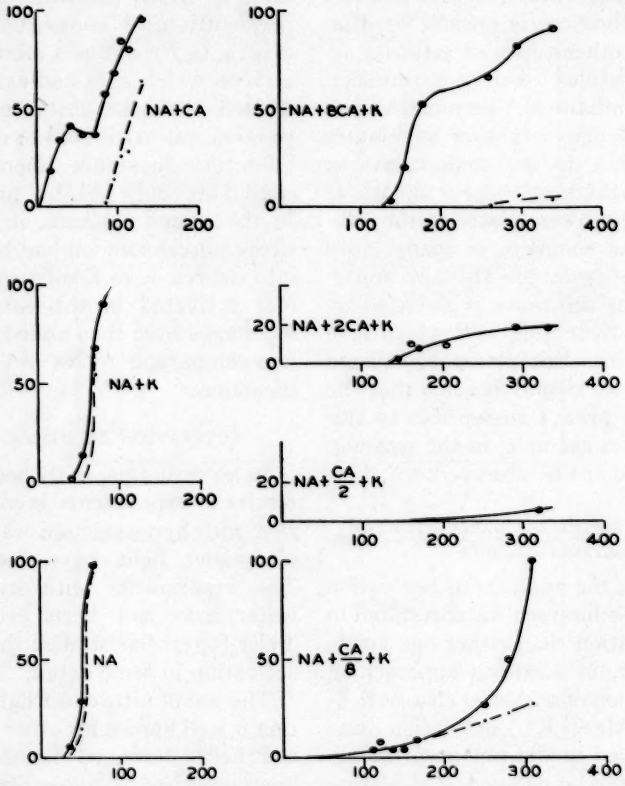

FIG. 5.—Activation of *Urechis* eggs in mixtures of sodium sea water with isotonic potassium chloride and calcium chloride solutions. Potassium concentration as in sea water, calcium concentration variable. Symbols as in Fig. 2.

water was drawn off, more eggs were added and irradiated. This was repeated once more. Eggs were then added again to the water in which three different lots of eggs had previously been activated (dish 1). At the same time, eggs were also added to a second dish of water which had been carried through the same procedure of irradiation, filtration, etc., but without eggs (dish 2). The two dishes were irradiated, with the result that 47 ± 3.2 per cent of the eggs in dish 1 were activated, but only 7 ± 1.9 per cent of those in dish 2.

This unexpected result led to a second experiment, with three samples of sea water. To the first, eggs were added, irradiated, and the eggs filtered out. This was repeated four times. The second sample was treated in exactly the same fashion, but without eggs. In the third sample the eggs were added and filtered out *before* irradiation. Finally, eggs were added to all three samples of sea-water and irradiated. The percentages of activation were: (1) 1.3 ± 0.6 per cent; (2) 3.1 ± 0.75 per cent; (3) 47 ± 2.8 per cent. A second irradiation was given after 1 hour, with the following results: (1) 15 ± 2.2 per cent; (2) 6.7 ± 1.3 per cent; (3) 100 per cent. These results indicate (*a*) that water from unfertilized

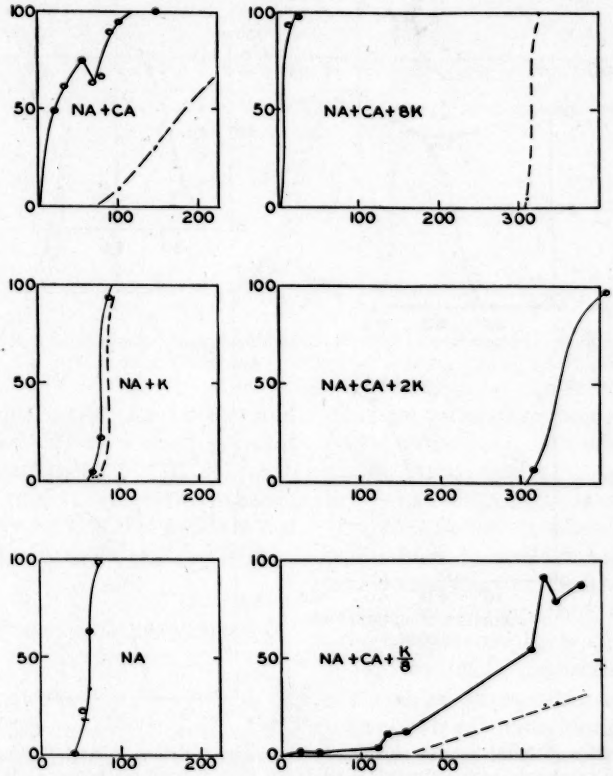

FIG. 6.—Activation of *Urechis* eggs in mixtures of sodium sea water with potassium and calcium chlorides, with varying potassium concentrations. Symbols as in Fig. 2.

eggs (3) contains a substance which facilitates activation and (*b*) that water in which eggs have been activated (1) contains less of this substance or more of an inhibitory substance. It appears possible that the facilitating substance might be fertilizin (F. R. Lillie, 1919; Tyler, 1941*b*). Allee and co-workers (1942) have shown that the early cleavages of fertilized *Arbacia* eggs are accelerated by a substance present in fertilized eggs. The acceleration of cleavage by crowding, which they reported earlier, may in part be caused by this substance.

Studies of the time course of ultraviolet activation confirmed the facilitating effect of egg water (Fig. 7). Eggs were placed in sea water in a finger bowl under the lamp and stirred continuously with a pipette. At 15-second intervals samples were withdrawn to Syracuse dishes containing sea water. After an appropriate period, the number of eggs activated was counted in each dish. The curves in Figure 7 represent the percentage of eggs activated by irradiation for the period of time (in seconds) indicated. Activation was more rapid in water in which eggs had previously been activated (*B*, *D*) or in washings from unfertilized eggs (*E*) than in fresh sea water, but no evident difference appeared between water from activated and unactivated eggs.

The observations of Heilbrunn and

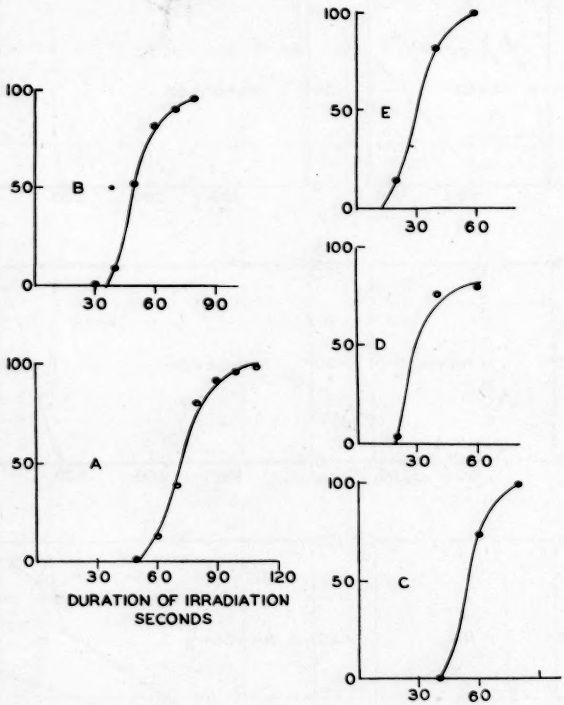

FIG. 7.—Activation of *Urechis* eggs in sea water by ultraviolet radiation. *A*, fresh sea water; *B*, sea water in which eggs had previously been activated; *C*, different eggs in fresh sea water; *D*, in sea water from activated eggs; *E*, in sea water from unactivated eggs. *Abscissa*, duration at irradiation in seconds; *ordinate*, percentage of eggs activated.

Wilbur (1937) concerning the role of calcium in ultraviolet activation of *Nereis* eggs have been repeated with eggs of *Urechis*. Treatment with isotonic sodium citrate temporarily prevented activation; addition of citrate to sea water retarded activation. In one experiment, 40 seconds were required for activation by ultraviolet of 50 per cent of a particular sample of *Urechis* eggs in sea water. Similar eggs, treated for 10 minutes in a mixture of equal parts of sea water and isotonic sodium citrate, were 50 per cent activated after only 95 seconds of irradiation. A few eggs (14 per cent) were activated merely by transfer to sea water from the citrate mixture (cf. Wilbur, 1941).

served to inhibit activation, by any of several methods, in eggs of both *Urechis* and *Strongylocentrotus purpuratus*. In this earlier work eggs were treated for definite periods of time, with and without additions of the drug, and then counted after an interval. In most of the work to be reported here, the influence of the drug on the time course of activation was followed.

When acetylcholine was added to sodium sea water or sodium sea water with added potassium, the effect was very slight. Activation and cytolysis were somewhat delayed in a few instances (Fig. 8). However, when acetylcholine was added to sodium sea water with added calcium, the result was more dramatic; the calcium effect, when this was present, was completely eliminated (Fig. 8). Instead of the immediate activation often noted in mixtures of sodium sea water with calcium chloride, activation was considerably delayed. The effect of acetylcholine in this respect closely resembles that of potassium ion in low con-

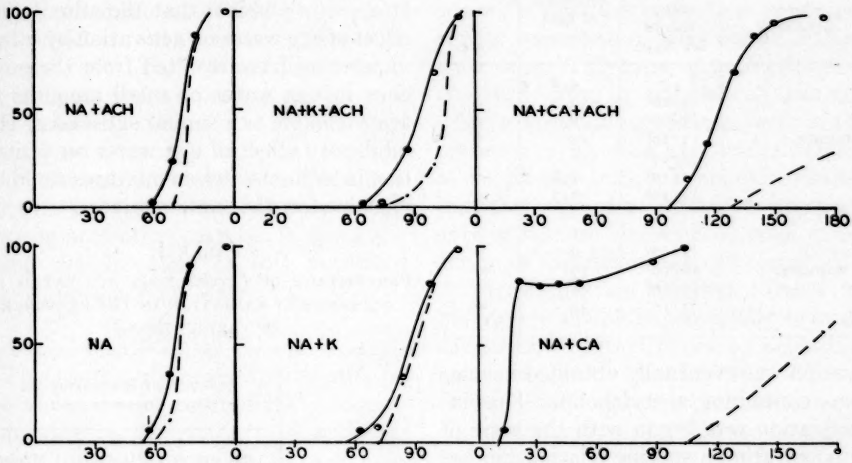

FIG. 8.—Effects of acetylcholine (1/10,000) on activation of *Urechis* eggs in isotonic solutions. Time in minutes.

THE EFFECTS OF DRUGS ON ARTIFICIAL ACTIVATION

ACETYLCHOLINE

In an earlier report (Scheer, 1945) data were presented showing that acetylcholine or physostigmine, at a concentration of one part in 10,000 of sea water,

centrations and of washings from eggs (Figs. 4, 6). Rapid activation in Na + Ca + 10 K mixtures was inhibited in the same manner by acetylcholine. It should be emphasized that this is not a toxic action, since perfectly normal activation was eventually obtained in solutions containing acetylcholine. This investigation was begun with the hope of demonstrating a specific relationship between calcium ion and acetylcholine. In view of the report of DuBois and Potter (1943) concerning the activation of adenosine triphosphatase, however, it was expected that this relationship would be one of synergism, not of antagonism. It appeared possible that the antagonism observed might be the result of the relatively high concentration (physiologically speaking) of acetylcholine used, since the actions of such high concentrations of acetylcholine on mammalian nerve cells are opposite in effect from those of lower concentrations (Goodman and Gilman, 1941).

TABLE 2

EFFECT OF ACETYLCHOLINE IN VARIOUS CONCENTRATIONS ON ACTIVATION OF *Urechis* EGGS BY ULTRAVIOLET LIGHT

Time Irradiated	Drug Concentration in Sea Water	Percentage of Eggs Activated	Difference from Sea Water
1 minute	0	5.1	.....
	1/10⁴	2.1	0
	1/10⁵	28	+
	1/10⁶	20	+
	1/10⁷	28	+
	1/10⁸	6.8	0
	1/10⁹	6.7	0
1½ minutes	0	21	.....
	1/10⁴	0	−
	1/10⁵	19	0
	1/10⁶	46	+
	1/10⁷	49	+

Unfortunately, this idea occurred only late in the spring, at which time the typical calcium effect was not often observed. However, the results of preliminary experiments indicate that acetylcholine inhibited activation in sodium-calcium mixtures even when the drug was diluted to one part in 10^9.

The same is not true, however, for the action of acetylcholine on ultraviolet activation (Table 2). With ultraviolet radiation, concentrations of acetylcholine below one part in 10^5 appeared to be stimulatory, while concentrations of one part in 10^4 and higher were inhibitory. It appears possible that the stimulating effect of egg water on activation by ultraviolet may have resulted from the presence in egg water of small amounts of acetylcholine or a similar substance. The inhibitory effect of egg water on activation in sodium-calcium mixtures could be explained in the same manner.

TABLE 3*

PERCENTAGE OF *Urechis* EGGS ACTIVATED BY ULTRAVIOLET RADIATION IN THE PRESENCE OF VARIOUS DRUGS

Solution	Duration of Irradiation and Drug Concentration		
	1 Minute 1/10,000	2 Minutes 1/10,000	1½ Minutes 1/100,000
Sea water	17	99	45
Adrenaline	3.5−	2.3−	11 −
Pilocarpine	2.0−	3.3−	31 0
Atropine	5.9−	99 0	8.7−
Arecoline	4.0−	15 −	11 −
Nicotine	92 +	97 0	85 +
Histamine	0.7−	5.4−	56 0
Physostigmine	96 +	81 −	98 +
Choline	5.3−	15 −	15 −
Acetylcholine	3.5−	8.7−	16 −

* Significant differences from sea water are indicated by + or −.

OTHER DRUGS

In addition to acetylcholine, a number of other drugs were tested with ultraviolet irradiation. The results are summarized in Table 3. Fresh solutions were prepared, immediately before use, from the following drugs: adrenaline chloride

solution (Parke-Davis, contained chloretone and bisulphite); pilocarpine hydrochloride; atropine sulphate, hypodermic tablets; arecoline bromide; nicotine alkaloid; histamine phosphate; physostigmine salicylate; choline hydrochloride; acetylcholine bromide.

It is apparent from Table 3 that the inhibitory action of acetylcholine on activation was not specific but was shared by a number of drugs of varied composition. It was earlier reported that physostigmine had effects similar to those of acetylcholine, with certain differences (Scheer, 1945). The results of the present experiments make it necessary to revise this statement. The effects of physostigmine were irregular, and, in the absence of more extensive data, we cannot say with certainty what the action of this drug on activation may be. It should be noted that nicotine ($1/10^4$) activated eggs in ordinary sea water.

DISCUSSION

The results of these experiments, on the whole, do not contradict the suggestion made earlier—namely, that liberation of calcium from an intra-cellular compound, activation of adenosine triphosphatase, movement of potassium across the cell membrane, and formation or liberation of acetylcholine are all important in excitation. In particular, the results provide evidence of a relation between calcium ion and acetylcholine in activation of *Urechis* eggs.

We may distinguish two types of activation in the experiments reported here. The first was characterized by a relatively long latent period, in that activation appeared only at some time after the activating treatment was initiated. This type of activation resulted from the action of sperm, ultraviolet light, hypotonic sea water, or isotonic salt solutions lacking calcium ion. It also was observed in the presence of calcium ion in the summer months or when acetylcholine was present. We have suggested that this type of activation resulted from an indirect action of the stimulating agent; on the basis of Heilbrunn's (1937) theory, we may suppose that the actions of such an agent will include breakdown of a cortical calcium compound.

The second type of activation was characterized by a very brief latent period, with signs of activation appearing almost immediately after the activating treatment. This type of activation was observed only in unbalanced isotonic solutions containing calcium ion or in the presence of nicotine at a concentration of one part in 10,000. It appears possible that this type of activation, in the presence of calcium ion, resulted from direct action of this ion on the cell cortex. The action of nicotine deserves further inquiry. One would like especially to know the action of acetylcholine on activation by nicotine.

The antagonistic relation between calcium ion and acetylcholine has been noted above. It appears worth while to consider the possibility that competitive inhibition of an enzyme system such as adenosine triphosphatase may be involved here. If we accept the results of DuBois and Potter (1943), we may imagine that the activation of the adenosine triphosphatase of mammalian muscle by calcium or acetylcholine involves a combination of these ions with the enzyme. It is then conceivable that acetylcholine might combine with other phosphatases, but in such a manner as to prevent the activation effect of calcium ion, without itself activating the enzyme. An objection to this explanation lies in the fact that in our experiments acetylcholine only inhibited the specific activating effect of calcium ions; activation in other

unbalanced solutions was only slightly delayed by acetylcholine. If all activations involve internal liberation of calcium ions, as Heilbrunn suggests, we might expect complete inhibition of all types of activation by acetylcholine. A second phenomenon, which does not easily fit the specific inhibition hypothesis, is the fact that low concentrations of acetylcholine facilitated activation with ultraviolet. Evidently, further examination of possible mutual interactions of acetylcholine, calcium ion, and adenosine triphosphatase is required.

Another possibility is that acetylcholine prevents the action of calcium ion by preventing its diffusion into the egg. We have made a number of determinations of the rate of cytolysis of eggs of *Urechis* and other marine species in diluted sea water and have found no effect whatsoever of acetylcholine. It evidently has no influence on permeability to water. However, there are slight indications in the experiments reported earlier that acetylcholine reduced somewhat the permeability of *Urechis* eggs to cations. Thus activation, and especially cytolysis, were sometimes delayed by addition of acetylcholine to sodium sea water or sodium sea water and added potassium (Fig. 8). It is conceivable that acetylcholine is more especially concerned with permeability to calcium ions. The fact that high concentrations of potassium ion increased the rate of activation in sodium-calcium mixtures is also of interest here, suggesting possibly that such high potassium-ion concentrations increase permeability to calcium ions. Alternatively, we might suppose a direct action of potassium ion in high concentrations, which would summate with the effect of calcium ions.

In several instances evidence was present that substances influencing artificial activation were liberated into the water by eggs. This was apparent in the effects of crowding and in the effects of solutions in which eggs had been activated previously or in which unactivated eggs had been washed. Crowding, in all instances, depressed activation. This might be attributed to accumulation of metabolites or to other factors; we have not examined the phenomenon in detail. It is of special interest that the effects on activation by Na + Ca of crowding or of solutions in which eggs had been activated were identical with those produced by adding acetylcholine to the solution. Activation by ultraviolet was facilitated by egg water, although crowding depressed activation; this is of interest, in view of the facilitating effects of small concentrations of acetylcholine. Activation in Na + 10 K was also facilitated by washings from activated eggs. Our data do not permit critical examination of the phenomena, but the problems raised would appear to deserve further examination.

SUMMARY

1. Eggs of *Urechis caupo* were activated by immersion in a modified artificial sea water containing sodium as the only cation.

2. Activation in this solution was closely related to cytolysis; both phenomena are attributed to a disturbance of the ionic balance of the egg.

3. Addition of other cations to this solution retarded the activation, with two notable exceptions.

4. High concentrations of potassium ion increased somewhat the rate of activation; cytolysis was retarded.

5. Low or moderate concentrations of calcium ion often increased the rate of activation very markedly and somewhat retarded cytolysis.

6. High concentrations of potassium ion increased the accelerating effect of calcium ion.

7. Acetylcholine did not greatly influence activation or cytolysis of eggs in sodium sea water or in sodium sea water with added potassium or magnesium.

8. Acetylcholine completely inhibited the specific effect of calcium in increasing the rate of activation.

9. Irradiation with ultraviolet light in sea water resulted in activation of *Urechis* eggs.

10. Acetylcholine in low concentrations ($1/10^6$ or less) increased the effect of ultraviolet; in higher concentrations ($1/10^4$) acetylcholine was inhibitory.

11. Nicotine $1/10^4$ in sea water activated *Urechis* eggs.

12. Adrenaline, pilocarpine, atropine, arecoline, histamine, or choline, $1/10^4$ in sea water, inhibited activation by ultraviolet.

13. These results are discussed briefly in relation to the suggestion that calcium ion, potassium ion, adenosine triphosphatase, and acetylcholine are important in excitation generally.

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THE EFFECTS OF CERTAIN DENATURED ORGANS ON NEURALIZATION IN AMBLYSTOMA EXPLANTS

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THE classical example of a second-grade organizer is the development of the lens as a result of the contact between optic cup and overlying ectoderm. That the mechanism might involve the transfer of a chemical substance was first suggested by Lewis (1904), but the effect of dead optic cup on competent ectoderm was first reported by Lopashov (1935, 1936). He obtained retinal tissue, nearly always in association with induced brain rudiment;

occasionally, small lenses were induced in the overlying ectoderm. These results were interpreted as evidence of a specific inductive effect of the dead optic cup. Similar results were obtained by Van Cleave (1938). In neither of the above series of experiments were lenses induced directly by the dead optic cup; such lenses as were obtained were obviously the result of an induction effected, in turn, by the optic cup associated with an induced brain rudiment.

The present investigation is concerned primarily with the specificity of the inductive effect of certain denatured material. Since, as seen above, the induction of retinal tissue, even if usually associated with other brain rudiments, was interpreted as a specific inductive effect of the dead optic vesicle, it was essential that these results which were obtained with one organ rudiment be compared with the possible inductive effects of other discrete embryonic organs or regions. This series of experiments comprises a comparison of the possible inductive effects of dead optic cup, dead hindbrain material from the same embryo from which the eyecups were taken, and dead larval liver tissue. All material and controls were tested for induction in isolated ectodermal explants from various stages of gastrulation of *Amblystoma punctatum* and *A. tigrinum*.

The use of isolated explants of competent ectoderm for the assay of induction originally seemed a nearly ideal procedure, as compared with the alternative of placing the material to be tested within the blastocoele of a host embryo. It avoids the complications introduced by possible "field" effects of the host and by the presence of mesoderm. The physiological adequacy of the culture medium is indicated by the survival of explants sufficiently long for them to achieve histological differentiation. Fundamental to the validity of this technique is, of course, the reliability of the supposition that, in the absence of inductive stimuli, the presumptive epidermis of the early gastrula is incapable of forming neural tissue. From his unparalleled experience in explanting gastrular ectoderm from a wide variety of anuran and urodelan species, excepting *Amblystoma*, Holtfreter could conclude that among hundreds of explants not a single case was found which proved with certainty that presumptive medullary plate, isolated in the gastrula state, could develop neural tissue independent of an inductor (Holtfreter, 1938). This lack of determination of the early gastrular ectoderm has been widely accepted as a well-established fact.

However, Barth (1941) presented evidence that isolated gastrular ectoderm of *A. punctatum* formed neural tissue in the absence of inductor. Holtfreter (1944) confirmed this exceptional behavior and, on analysis (1945), concluded that the inductors in this case were certain substances released as a result of cytolysis of cells of the inner uncoated layer of the ectoderm, caused by exposure to the standard Holtfreter saline solution. The unique capacity of explanted *Amblystoma* ectoderm is presumably causally related to the unique susceptibility of this ectoderm to the inorganic medium; and the resultant differentiation of neural tissue falls into line with induction of neural tissue obtained with the other amphibian explants.

Although one can agree with Holtfreter and Barth that all experiments with dead inductors or with foreign tissues must be reconsidered critically from the viewpoint of cytolysis, it is not clear that the explant technique is wholly without value. It should be emphasized that Barth's original discovery and Holtfreter's analysis of the factors causing

disintegration of the inner layer of the ectoderm concerned isolated strips or pieces of gastrular ectoderm, which were allowed or, in some cases, compelled to fold or roll up into a ball of ectoderm. Barth (1941) found that the fusion of two explanted pieces of ectoderm by their inner plane surfaces inhibited formation of neural tissue. His Table 5, giving the results of strictly comparable experiments on end-to-end fusion and on surface fusion, shows no neural differentiations occurring in the later explants in *A. punctatum*. Holtfreter (1944, 1945) shows in a number of ingenious ways that prolonged exposure to standard solution affects the inner, uncoated layer of ectoderm cells and that, if this inner cell layer is prevented from remaining in contact with the medium, the percentage of explanted pieces of ectoderm showing neural differentiation drops from 60–70 to 15 per cent.

Another factor which Holtfreter found effectual in altering the percentage of neural differentiation in unprotected pieces of ectoderm was the pH of the medium. The usual pH of the standard Holtfreter solution is 8.2; when this was acidified to produce pH 6.4–7.0, 10.8 per cent of the explants showed neural tissue, as compared with the 60–70 per cent in the controls. This suggests that an acid medium is unfavorable to neural induction, although the mechanism involved is unclear.

MATERIAL AND METHODS

In these experiments the inductive capacity of dead material was tested by the method of explantation exclusively. The material to be assayed was placed between two pieces of ectoderm of the same size and shape, which quickly fused to form an unbroken outer coat of ectoderm. The ectoderm used came chiefly from gastrulae of *A. punctatum;* a supplementary series of similar experiments was done with ectoderm of *A. tigrinum* and is referred to in a separate section below.

In preparing all ectodermal explants, particular care was taken to specify the exact stage of gastrulation reached by the donor egg. The following stages, corresponding to Harrison's series, are referred to: Stages 9, 10 (slit blastopore), 11 (horseshoe-shaped blastopore), early 12 (large-to-medium yolk-plug), 12 plus (small yolk-plug), and 12½ (slit blastopore, no yolk-plug).

After orienting the egg, a small area of ectoderm, 180° opposite the blastopore, was removed and always carefully cleaned, if necessary, of adherent cells. In the earlier stages of gastrulation, this ectoderm was presumptive belly epidermis plus presumptive anterior medullary plate; with advancing gastrulation, the ectoderm was taken from the area of presumptive epidermis only. In all explants, both top and bottom pieces of ectoderm came from the same egg. All operations were done with a sterile technique. The medium used for operation and isolation was Holtfreter's 0.35 per cent saline solution *without* $NaHCO_3$ and with spring water substituted for distilled water. The original pH of this medium was 7.2–7.4, but during the period of isolation it rose to 7.6.

The material whose inductive capacity was to be tested was prepared as follows: the optic vesicles of *A. punctatum* embryos (Stages 20–21) were cleanly removed from the diencephalon and dropped at once into 95 per cent alcohol. When the last vesicle of the particular lot of embryos had been added, the alcohol was drawn off and the vesicles inspissated at 60° C. for 1–18 hours. Variable periods of time elapsed before this material was placed in explants; this can best be mentioned below. The so-called

"hindbrain" material consisted of the rest of the brain from a level just caudal to the diencephalon to a level between somites 3 and 4, taken from the same neurula as the vesicles or from another of the same stage. The brain piece was cleaned of adherent cells and placed in 95 per cent alcohol for a few minutes, after which the alcohol was removed and the material dried like the vesicles. Before it was used, the hindbrain was divided into pieces of approximately the same volume as one optic vesicle. The livers were removed from *A. punctatum* larvae of 35-mm. all-over length. After being washed in sterile water, the liver was placed in 95 per cent alcohol for about 1 minute, the alcohol then removed, and the liver inspissated for 4 hours at 60° C. Like the hindbrain, the liver was divided into smaller pieces before it was used. In addition to the killed material, living eye vesicle, hindbrain, and liver from embryos of the same developmental stages as above, which had been sterilized by repeated washings, were also used in explants. In the main body of the work, approximately 50 explants were made with each of the three testing materials in each of four gastrular stages up to the close of gastrulation.

HISTOLOGICAL CRITERIA

Explants of isolated ectoderm on microscopic examination present one of two pictures: either the mass of tissue is wholly epidermal, or neural tissue in some degree of histological and organological differentiation is also present. Nonectodermal tissues are not found. The differentiation of neural tissue ranges from a type of "palisade" or "neuroid" tissue through histologically more objectively neural tissue, which apparently lacks the capacity to form organotypical structure, to neural tissue, which forms brain masses and diverticula with or without associated sense organs, such as nasal placodes and optic cups. The neuroid type is characterized by the elongation and lining-up of cells of the otherwise epidermal mass, perpendicular to the contacting dead material (Fig. 4). In most cases the staining reaction of the neuroid cells contrasts with that of the surrounding epidermal mass. Neuroid tissue is here considered as the lowest grade of neural induction. In some instances a confusion exists between neuroid tissue and merely thickened and stratified epidermis; doubtful cases were recorded as epidermis. Neuroid tissue appeared most frequently in otherwise epidermal explants lying immediately in contact with the implant. Sometimes one such neuralized area led directly into an adjacent neural ball or vesicle; neuroid tissue was never found associated in the same explant with an organotypical neural tissue. If neuroid tissue appeared only in explants killed after a short period of isolation, there might be grounds for believing it to be an early developmental stage of more highly organized neural structures or the explant equivalent of a medullary plate. But it also appears in explants isolated 10 days or more, a period which is more than sufficient for the development of very elaborate brain diverticula.

An assumed higher type of differentiation is seen in neural tissue, the neural character of which is histologically clear and definite but which failed to combine into formed structures. It consists typically of masses, usually spherical, of neural cells, irregularly disposed and usually sharply delimited from surrounding epidermis. Internal cavities, slits, or crevices, such as might be formed by uniform orientation of cells, were lacking. Examples of this type were never common in any series of explants.

Organotypical neural tissue varied in

size and volume from short neural-tube-like structures, small in relation to the mass of surrounding epidermis, to brain masses representing the complete transformation of the original explanted ectoderm into neural tissue, with a uniform high order of differentiation and organization (Figs. 2, 3, 5, 6). Associated with such thick-walled brain masses might be one or more optic vesicles and nasal placodes (Fig. 6). Orderly or symmetrical regional organization of these masses, such as would imply the existence of an embryonic axis, was not observed. The impression given by one of these masses is, rather, one of mechanically imposed crumpling and distortion of potent but undirected organ differentiation, combined with a surprising degree of histological differentiation. Mesenchyme was occasionally associated with such structures, melanophores less frequently. From such observations one can agree with Holtfreter's (1944) conclusion: "Isolated gastrula ectoderm of any derivation tends to form anterior head-structures. The differentiation is the more organotypical the more neural tissue is involved."

The usual "sandwich"-type explant, which was made by allowing two pieces of ectoderm to fuse with their inner surfaces in contact and which contained no implant of any kind, constituted the chief type of control experiment. Such ectodermal controls were usually prepared alternately with experimental explants from a given lot of eggs of each stage used and were subsequently given identical treatment and isolation. The microscopic examination of scores of such ectodermal controls from Stages 9, 10, and 11 has uniformly revealed only epidermis. Under the routine experimental conditions, by which the inner ectodermal coat is only shortly exposed to saline solution and in the absence of heterogeneous cells or materials, explants of presumptive epidermis and of presumptive anterior medullary plate differentiated as pure epidermis.

In explants of ectoderm from a late stage of gastrulation, neural tissue was encountered. In a series of 31 explants from Stage 12, 29 differentiated as epidermis, 1 showed neuroid tissue, and 1 a small neural mass without structure. Of 62 explants carefully oriented as presumptive belly epidermis from Stage $12\frac{1}{2}$, all were purely epidermal, with no trace of neural tissue. Approximately half the explants of this series had been isolated for 2–3 weeks at 5°.6 C. for other purposes.

Another type of ectodermal explant was made in the attempt to duplicate if, possible, with the explant technique, the conditions surrounding implants in the blastocoele cavity. Very small miniature explants were made of Stage 10 ectoderm, and each was allowed to fuse firmly as usual. After 3 hours, such a miniature explant was placed as an implant within an explant of Stage 11 ectoderm, made slightly larger than usual. Other miniature explants were similarly used as implants after 24 hours' isolation. Both sets of explants with contained implants were killed after 8 days' cultivation. In all 20, epidermis only was found; and in only 2 explants did the implant survive as a solid epidermal ball, lying within a cavity surrounded by epidermis. In each of the other explants a discrete mass of disintegrated cells or cellular debris, usually lying within a central cavity, represented the implant. The experiment is interesting in view of the suggestion that has been made that products of necrosis released by cells that have been killed by various implanted substances are capable of acting as inducing stimuli. In this series neither the living cell masses nor the cellular debris produced

any change in the presumptive fate of the contacting ectoderm.

EXPLANTS CONTAINING DEAD OPTIC CUP

In Table 1 are recorded the results of microscopic study of 206 explants which contained dead optic cups. The ectoderm tested in these explants came from many different bunches of eggs from several geographical sources. To give the full group of explants consisted of 7 separate groups done at various times, using optic cups, which had been kept, respectively, 1 day, 10 days, 52 days in 3 groups, 2 months, and 9 months. No correlation between age of implant and amount and character of induction was observed.

Table 1 shows that ectoderm from four stages of gastrulation, which was

TABLE 1

NEURALIZATION IN EXPLANTS WITH DEAD OPTIC CUP

	Epidermis	Neuroid	Unorganized Neural Mass	Brain Mass	Totals
Stage 9:					
Number	31	10	7	9	57
Per Cent	54.3	17.5	12.3	15.8	.....
Stage 10:					
Number	11	9	8	19	47
Per Cent	23.4	19.0	17.0	40.4	.....
Stage 11:					
Number	12	14	4	26	56
Per Cent	21.5	25.0	7.1	46.5	.....
Stage 12 (early):					
Number	5	12	4	25	46
Per Cent	10.9	26.1	8.7	54.3	.....
Stages 9–12 incl.:					
Number	59	45	23	79	206
Per Cent	28.6	21.8	11.2	38.3	.....

protocols is impracticable; no group of explants of the same age and subjected to the same treatment gave results which could not be interpreted as normal variation from the summarized picture. A further assumption as to the uniformity of the implanted material also seemed warranted by the results. The dead optic cups used as implants varied, in respect to the period from the time of removal and killing in alcohol to the time of implantation, from 24 hours to approximately 1 year. In each group of explants, dead vesicles kept for different periods were used. For example, the Stage 10 subjected to the influence of dead optic cup, increased its response with advancing development. This is shown in two ways—first, while 54.3 per cent of the explants of Stage 9 ectoderm realized their prospective fate, unaffected by the implant, and developed as pure epidermis, only 10.9 per cent of the early Stage 12 explants remained unaffected; second, while only 15.8 per cent of the Stage 9 explants showed neural tissue of of the organizational level of neural tubes and brain masses, 54.3 per cent of the Stage 12 explants showed such response. The neuroid response increased

only slightly, perhaps insignificantly, throughout the developmental stages recorded. Explants showing neural tissue without recognizable structural organization decreased as gastrulation advanced. Perhaps the distinction made on the presence or absence of recognizable structure is artificial and both should be recorded simply as histiotypical neural tissue.

Of 206 explants of presumptive epidermis exposed to dead optic vesicle, 127, or approximately 71 per cent, responded with some degree of neural differentiation. Exclusive of the neuroid response, 102 explants, or 50 per cent of the total number, showed unmistakable neural tissue. But, of this number, only 6 explants with brain masses presented the heavily pigmented tapetal layer with retina of such configuration that it could conservatively be called an "induced" optic vesicle. Other induced brain masses were of considerable size and complexity, sometimes involving the entire interior of the explant and occasionally erupting to the surface to lie exposed as if the covering epidermal layer had been an ineffectual barrier to its expanding development. The impression given by many of the larger brain masses was one of crumpling and distortion because of development within a confining space. Nevertheless, in spite of the presence of well-differentiated brain parts and considerable histological differentiation, in only 6 explants did this differentiation of parts produce a recognizable optic vesicle. In none of these 6 explants was there any evidence of bilaterality or of axiate regional organization. The induced optic vesicle was always a part of a larger brain mass of which it appeared to be a regional differentiation.

EXPLANTS CONTAINING DEAD HINDBRAIN

Table 2 records the results of microscopic study of the 214 sectioned explants which contained pieces of dead hindbrain roughly equivalent in volume to one dead optic vesicle. As in Table 1, attention is centered on the induction of neural tissue. Procedure as to preparation and treatment of explants was as similar to that used with the optic cup as possible.

The same diversity of ectoderm and of implant material obtained as described above. In a few instances both kinds of explants were prepared from the same bunch of eggs; this, however, was not generally the case. As with dead optic vesicle, no correlation was observed between age of implanted material and amount and character of induction.

The quality and character of the neural inductions obtained with dead hindbrain material did not differ from those obtained with dead optic cup. Certain quantitative responses given by ectoderm of the four stages of gastrulation compared did differ appreciably. Whereas the reactivity of the ectoderm in explants containing dead optic cup increased with advancing gastrulation, the reverse occurred with implants of dead hindbrain. Of 99 explants of Stages 9 and 10 ectoderm, none differentiated as pure epidermis; all showed some degree of neural response. With Stage 11 ectoderm, 21 per cent were epidermal, and this figure increased to 25 per cent with early Stage 12. The obverse of this picture of increased ectodermal response to dead hindbrain material is indicated in the production of brain masses with increasing age. Of Stage 9 explants, 59 per cent showed brain masses, as compared with 16 per cent in a similar eyecup series; but this high figure fell to 19 per cent with Stage 12 ectoderm, while the 16 per cent rose to 54 per cent in comparison.

Any comparison of the relative potency of hindbrain and optic cup must be confined to the relative response of ectoderm of the same stage of gastrula-

tion. With early ectoderm of Stages 9 and 10, hindbrain produced a total neural response superior in every respect to that produced by dead optic cup on ectoderm of the same age. It is interesting here that the neuroid response in both instances was almost exactly the same. But in ectoderm from later stages, more explants gave more neural response to eyecup than to hindbrain. When the above disappear, and significant differences of effect may be lost.

In this series of explants containing hindbrain fragments, an induced optic rudiment was positively identified in each of 3 explants, all made of older gastrular ectoderm. Each rudiment was a component part of a larger brain mass; associated with one were two lentoids (Fig. 6). The same possibility of failure

TABLE 2

NEURALIZATION IN EXPLANTS WITH DEAD HINDBRAIN MATERIAL

	Epidermis	Neuroid	Unorganized Neural Mass	Brain Mass	Totals
Stage 9:					
Number	0	7	11	26	44
Per Cent	.....	16.0	25.0	59.0	.....
Stage 10:					
Number	0	11	16	28	55
Per Cent	.....	20.0	30.0	50.0	.....
Stage 11:					
Number	12	18	16	12	58
Per Cent	20.7	31.0	27.6	20.7	.....
Stage 12 (early):					
Number	14	29	3	11	57
Per Cent	24.6	50.9	5.3	19.3	.....
Stages 9–12 incl.:					
Number	26	65	46	77	214
Per Cent	12.1	30.4	21.5	36.0	.....

four stages used are summarized, it is seen that hindbrain and eyecup produced almost the same total percentage of brain masses, although hindbrain material did excel in the production of what may be called "low-grade neural tissue" and did effect a neural response of some order in a greater number of explants.

It should be pointed out that, when explants of ectoderm from several stages of gastrulation are averaged together indiscriminately and comparison is attempted with another average similarly arrived at, such marked component differences and trends as were discussed to recognize an optic rudiment because of incomplete histological differentiation and distortion of form exists here as it did in the former series. However, in both series the number of such questionable structures was relatively small, and it seems unlikely that their failure to achieve more advanced development was due to too short a period of cultivation.

EXPLANTS CONTAINING FRAGMENTS OF LARVAL LIVER

Data from which any profitable discussion of specific inductive effects of dead material can emerge must neces-

sarily include observations on as wide a variety of such materials as possible. In the present work, liver tissue was chosen to serve as a suitable contrasting agent to the two ectodermal tissues. It was physically easy to handle and easily obtained in large quantities from the larval animal, as previously described. Table 3 records the results of microscopic study of the 164 explants examined which contained pieces of dead larval liver. These pieces were roughly equivalent in size to the pieces of hindbrain used or to one dead optic cup. Procedure duplicated that in the previous series as closely as possible. The age of the implanted liver, i.e., the period from completion of inspissation to time of implantation, varied from a few hours to 41 days, with most of the material being 2 days old. No correlation between this factor of age of implant and the results was apparent.

Under the experimental conditions used, hepatic tissue was found to be very ineffective as an inducing agent (Fig. 7). Only in the series of ectodermal controls did purely epidermal masses appear with such uniformity. In the few instances in which neural tissue did appear in the liver explants, the palisades and small masses of unorganized neural tissue were similar to these previously observed; no brain masses or sense organs were seen.

A small number of explants containing living liver tissue was studied. These explants were made in the usual way with Stage 10 ectoderm; the liver was taken from Stage 44 animals, cut into four equal parts, and each quarter used as an implant after being washed in sterile saline. Fourteen explants were in excellent condition at the end of 7 days of isolation, at which time they were prepared for study. The liver implant was alive at this time in 8 of the explants and was represented by a mass of necrotic tissue in 5 others. Neural tissue in the form of palisades was present in 3 of the explants containing living liver tissue; its presence was either questionable or

TABLE 3

NEURALIZATION IN EXPLANTS WITH DEAD LARVAL LIVER FRAGMENTS

	Epidermis	Neuroid	Unorganized Neural Mass	Brain Mass	Totals
Stage 9:					
Number	46	0	0	0	46
Per Cent	100.0				
Stage 10:					
Number	39	5	2	0	46
Per Cent	84.8	10.9	4.3		
Stage 11:					
Number	35	6	2	0	43
Per Cent	81.4	13.9	4.7		
Stage 12 (early):					
Number	26	3	0	0	29
Per Cent	89.6	10.4			
Stages 9–12 incl.:					
Number	146	14	4	0	164
Per Cent	89.1	8.5	2.4		

definitely missing in the remaining 5, and also in the 5 explants in which the implant had died. The bulk of the explant was always epidermis. The living implant in each instance, and in most of those in which it was necrotic, was surrounded by mesenchyme-like cells which gave every appearance of having proliferated from the implant (Fig. 1). Blood cells could be recognized within or close to some implants; it was assumed that both mesenchyme and blood cells came from the living implant. In one explant from which the implant was extruded by the second day of isolation, nothing but epidermis differentiated.

EXPLANTS OF *A. tigrinum* ECTODERM

A relatively small number of experiments was made, using presumptive epidermis of *A. tigrinum* gastrulae of Stage 10. The procedure duplicated that followed with *A. punctatum* explants. Four series of explants were prepared: those containing implants of optic cup, hindbrain, liver, and ectodermal controls which contained no implant. The dead material used as implants had been prepared 22 days earlier from *A. punctatum* embryos. The ectoderm of the isolated explants in the living condition was more active than in *A. punctatum* explants, forming creeping processes which tended to anchor the explant to the lens-paper substrate. On microscopic study of the sectioned explants, a uniformly higher reactivity of the *tigrinum* ectoderm to inducing stimuli was reflected in the high frequency of neural inductions obtained.

Of 10 sectioned explants of *tigrinum* ectoderm which contained implants of *punctatum* optic cup, all were intensely neuralized. In most of them the entire mass of tissue comprising the explant was compact, well-differentiated neural tissue in the form of one large brain mass, whose most striking feature was the total lack of even a thin covering of epidermis. The mass of neural tissue lay naked and exposed. In all previously examined explants, at least a partial shell of epidermis was present, although in some instances it had apparently been broken through by the internal expansion of the developing neural mass. In only 3 of these *tigrinum* explants containing dead optic cup could any remaining nonneural tissue be found; this was, as in all the *tigrinum* series, epidermis. In none of these 10 explants had the implanted vesicle been extruded; in each it was completely imbedded in the brain mass.

Of the 9 sectioned explants of *tigrinum* ectoderm which contained fragments of hindbrain material, 7 showed the intense neuralization described above, while 2 were of a lesser order, although they exhibited a large brain mass. A covering epidermal layer was present in most of these 9, although the neural mass often erupted to the surface. The implant was solidly imbedded in each.

None of the 10 explants containing dead liver exhibited neural tissue in the amount and quality described above, although 1 explant contained a small brain mass, 5 showed neural tissue in the form of short neural tubes, and 1 an area of neural cells of no apparent organization. Three differentiated as epidermis only, but 2 of these had extruded their implants, and in the third the fragment of liver lay free in a large central cavity.

The ectodermal controls of this series of *tigrinum* explants consisted of 24 explants. Twelve were found to be purely epidermal, 7 showed unorganized areas of neural tissue, and 5 contained neural tissue in the form of small, irregular neural tubes. In comparison with the epidermal mass of the explant, the amount of neural tissue was extremely small, its

differentiation poor; but its staining characteristics and, in the case of the neural-tubelike structures, its structure marked it off from the mass of epidermis. Nevertheless, in the case of *tigrinum* ectoderm, one must admit that neural induction in the absence of inductor does occur in an appreciable percentage of explants. The strong neural response of the same ectoderm to eyecup and hindbrain inductors, and in a lesser degree to the liver material, makes it appear that *tigrinum* ectoderm, in comparison with *punctatum* ectoderm, is stepped up in its sensitivity or its response to inducing stimuli.

DISCUSSION

The primary object of these experiments was to test further the hypothesis of specific inductive effects. When the data from both *A. punctatum* and *A. tigrinum* explants are interpreted from this viewpoint, they lead to the conclusion that optic cup does not induce optic cup. Instead, the denatured optic cup called forth a response in the assay ectoderm which varied greatly in its quantity and intensity but which was always anterior neural tissue in quality. Very often the intensity of the response resulted in the formation of brain diverticula of considerable complexity and occasionally in the differentiation of indubitable optic vesicle. But the latter always seemed an expression of the intensity of the response and not a direct and specific response to the inductor. Nasal placodes were actually more common than induced optic vesicles, but they, too, appeared to depend on a certain amount and intensity of neural tissue's being first induced.

Also pointing toward nonspecificity of inductive effect are the results obtained with denatured hindbrain material. Again, only anterior neural tissue was induced, the quality of which was indistinguishable from that produced by dead optic cup, while the amount and intensity of organological differentiation varied as in the previous series. Where the two materials differed in their inductive effects was not in the quality of the inductions affected but in the developmental stage of the ectoderm at which they induced most strongly. This difference argues against the otherwise tenable supposition that both ectodermal materials are inductively alike when denatured by alcohol.

The third denatured material affords a strong contrast to the two ectodermal tissues. The results showed that larva liver, under the experimental conditions used, was relatively ineffective as a neural inductor or, in fact, as an inductor of any capacity, for the only induced tissue found in the otherwise epidermal explants was a little low-grade neural tissue. This was surprising in view of the work of Chuang (1939, 1940), who assayed the relative inductive capacities of both fresh and cooked mouse kidney and *Triton* liver in explants and as implants in the blastocoele. He concluded that his results proved that these heterogeneous organ parts were specific inductors, since mouse kidney induced chiefly brain parts, sense-organs, and other head structures, while *Triton* liver induced, above all, tail structures like muscles, chorda, and tail. This specificity was completely masked, however, when the materials were implanted in the blastocoele of intact animals. That both ectodermal and mesodermal structures were elicited in ectodermal explants in which mesoderm was lacking is especially noteworthy. Furthermore, the specific mesoderm-producing factor in *Triton* liver was more heat-labile than the ecto-

derm factor. Unfortunately, Chuang did not test the inductive capacity of cooked *Triton* liver in explants, so no direct comparison can be made between his results and ours. A comparison of the results due to the difference in method of denaturation would be interesting; certainly, denaturation by alcohol gives results which admit no clue as to the existence of a mesoderm-producing factor.

But in view of the work of Barth and of Holtfreter, an entirely different interpretation must now be considered. All the heterogeneous materials previously used as supposed inductors—fresh, denatured, or chemical—may produce their effects, not directly but by first injuring or killing the cells of the inner coat of ectoderm, which, in turn, release a substance or substances which effects the actual induction. As applied to the results under discussion, where all inductions are qualitatively alike in that only neural tissue was induced but in a differing range of intensity from "weak" to "strong," this view appears generally plausible. Both in quality and in quantity the inductions obtained with dead optic cup and dead hindbrain material closely resemble those obtained by Holtfreter when he subjected the inner coat of *Amblystoma* ectoderm to the noxious effect of standard saline solution. It seems clear, however, from my series of ectodermal controls, which only rarely (and then only in the late stages of gastrulation) differentiated as anything but epidermis, that the noxious agent in my series of experimental explants cannot be the culture medium but must originate in the implant itself.

Some experimental results do not fit readily into the above pattern. To explain the weak inductive capacity of denatured larval liver, one assumes that nothing emanates from the liver fragment that is more than mildly injurious to the sensitive inner ectodermal coat. If, as seems likely a priori, the dry fragment of liver does effect a change in its environment when wrapped in ectoderm this alteration does not, as a rule, effect the differentiation of the prospective epidermis. To be effective, the alteration must apparently be harmful. If larval liver is relatively innocuous, the cells of the embryonic central nervous system must, on denaturation, release a substance which is effectively injurious to the explant cells. This hypothetical noxious substance could hardly be identical in both dead optic cup and dead hindbrain, for the above data show induction to differ significantly according to stage of gastrulation in the two series.

In regard to the possibly noxious quality of the medium in which explants are operated on and cultivated, the evidence of explants containing no implant seems conclusive. It also seems clear from the liver series that mere mechanical contact, possibly involving pressure relations, bruising of cells, etc., is not effective as an inducing agent.

The results of the limited series of ectodermal explants which contained as implants smaller balls of similar ectoderm do not readily lend themselves to the injury hypothesis. In all but two cases the living implant disintegrated sometime during the period of isolation, for the sections show masses of cellular debris lying within the explant. Following Holtfreter's suggestion of a release of an inducing agent from the living cells upon necrosis, one might expect the requisite conditions for such release to be met in the above circumstances. But perhaps a necessary coincidence in time of stimulus and ectodermal competence was lacking.

Just as *A. punctatum* gastrula ecto-

derm is apparently uniquely different in its response from all other anuran and urodelan ectoderms which have been tested, so do differences exist within the genus. *Tigrinum* explants were characterized by the intensity of their neural response to all implants used and, in the case of ectodermal controls, to what may be considered the noxious effect of the culture medium even in protected "sandwich" explants. Here, again, the ectoderm responds to ostensibly widely different inductors—dead optic cup, dead hindbrain, dead larval liver—with qualitatively the same induction, which differs from that produced under similar circumstances in *punctatum* ectoderm only in greater quantity and intensity. Furthermore, the ectoderm in the complete absence of any implant shows an appreciable response in the same qualitative coinage, weak in intensity but still neural tissue. From this sequence of responses, one can conclude that *tigrinum* ectoderm is even more sensitive than is *punctatum* to noxious influences.

CONCLUSIONS

1. Denatured optic cup and hindbrain in isolated explants of *A. punctatum* gastrular ectoderm induce a qualitatively and quantitatively similar neuralization. They differ chiefly in the stage of gastrulation at which they are effective.

2. Denatured larval liver, as well as implanted fragments of living ectoderm which die during isolation, are weak or ineffective as neuralizing agents.

3. Control explants of ectoderm differentiate as epidermis only, except in rare instances of ectoderm from late stages of gastrulation.

4. The absence of neuralization in control explants and its absence or very low occurrence in various other series of explants, as compared with a high frequency with other implanted material or the same material in other stages of gastrulation, suggests that the inducing agent, whatever it may be, is not in the medium used but originates in the implant itself.

PLATE I

FIG. 1.—Photomicrograph of a section (×90) from explant AL2. Stage 10 *A. punctatum* ectoderm with an implant of living larval liver.

FIG. 2.—Photomicrograph of a section from explant TOV8 (×90). Stage 10 *A. tigrinum* ectoderm with implant of *A. punctatum* dead optic cup.

FIG. 3.—Photomicrograph of a section from explant F5-56 (×90). Early Stage 12 *A. punctatum* ectoderm with implant of dead optic cup.

FIG. 4.—Photomicrograph of a section from explant A12-10 (×90). Early Stage 12 *A. punctatum* ectoderm with implant of dead hindbrain. Neuroid tissue in immediate contact with implant.

FIG. 5.—Photomicrograph of a section from explant A30-10 (×90). Stage 9 *A. punctatum* ectoderm with implant of dead hindbrain.

FIG. 6.—Photomicrograph of a section from explant MA 25 (×90). Early Stage 12 *A. punctatum* ectoderm with dead hindbrain implant. Two lentoids associated with induced optic cup.

FIG. 7.—Photomicrograph of a section from explant A10L-6 (×90). Stage 10 *A. punctatum* ectoderm with dead liver implant.

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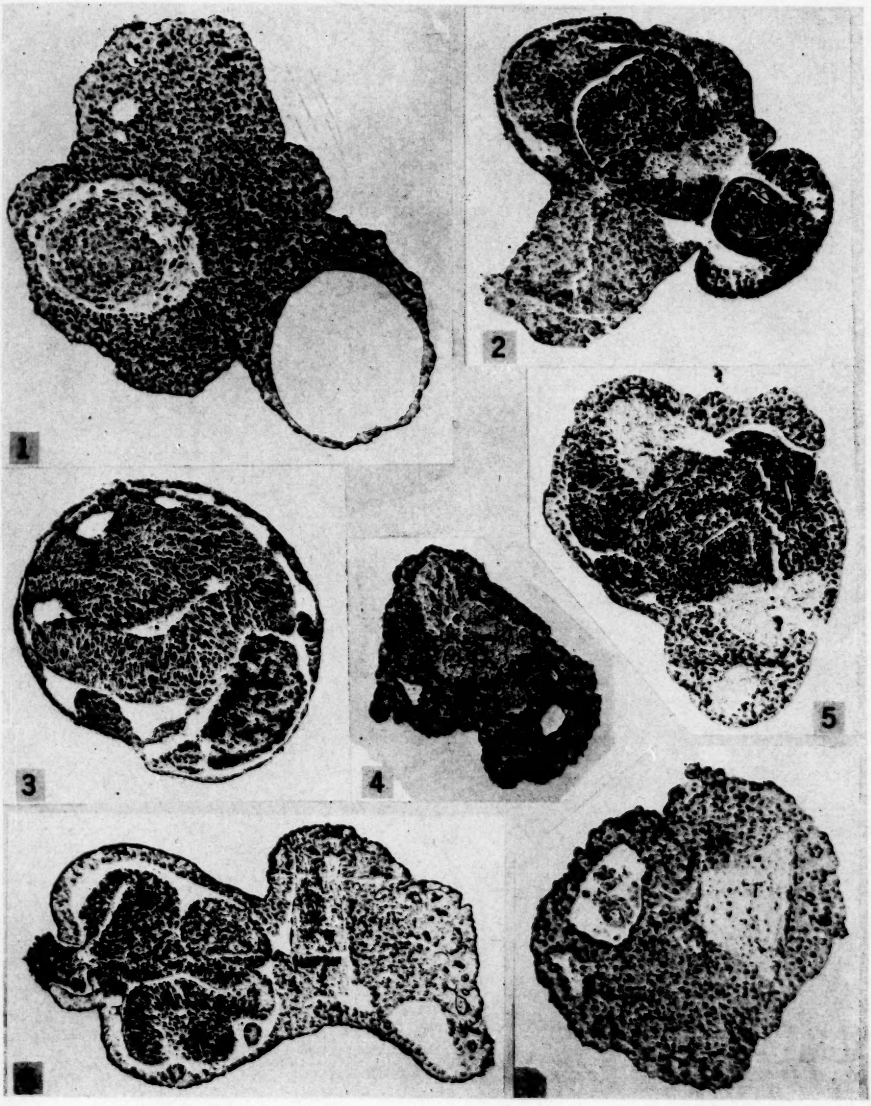
PLATE I



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THE POSTNATAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF THE GOLDEN HAMSTER (CRICETUS AURATUS) AND ITS REACTIVITY TO HORMONES[1]

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IN A previous study on the embryological development of the golden hamster (Ortiz, 1945), it was found that the reproductive system develops more rapidly in the hamster than in the rat or mouse. It was observed that, in spite of the much shorter gestation period (16 days), the reproductive tract of the golden hamster is as well developed at birth in most respects as that of the rat. In the present work the postnatal development of the gonads and accessory reproductive organs has been studied with especial reference to the rate of development and the age at which the organs are capable of responding to exogenous and endogenous hormone stimulation. Price and Ortiz (1944) concluded from their study of reactivity in the postnatal reproductive tract of the albino rat that the capacity to respond to hormone stimulation develops gradually. It is of particular interest to correlate the age and rate of development with the degree of hormone sensitivity in the hamster and to compare these factors in the prepuberal hamster and rat. For this purpose the present work has been undertaken.

MATERIAL AND METHODS

This research is based on the results obtained from 546 hamsters from the same stock as those used in the embryological study. Of these, 285 were injected, and 261 were kept as normal controls. A summary of normal body weights and organ weights of all the animals used for controls at each age appears in Table 1. With some exceptions, each litter was divided into approximately the same number of injected and control animals. Table 2 summarizes the number of animals in each injection series.

The hamsters were injected subcutaneously for 6 days with daily doses of 10 r.u. of equine gonadotropin, 0.1 mg. of testosterone propionate, or 1 r.u. (0.166 μgm.) of estradiol benzoate. The sex hormones were dissolved in sesame oil, and

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TABLE 1

SUMMARY OF NORMAL WEIGHTS

Age in Days	Females					Males					
	No. of Animals	Body Weight (Gm.)	Organ Weight (Mg.)			No. of Animals	Body Weight (Gm.)	Organ Weight (Mg.)			
			Ov.*	Ovd.	Ut.			Testis	V.P.	S.V.	C.G.
6	25	5.5	0.81	5.5		28	5.4	8.3	.....	.....	.....
10	27	8.6	1.2	1.1	9.0	31	9.1	19.3	0.3	1.3	0.4
16	22	17.5	2.1	3.9	18.0	22	17.1	51.3	1.0	2.8	0.6
26	26	40.5	14.6	7.0	56.2	22	37.2	290.0	5.6	13.6	2.7
36	26	46.1	17.4	10.1	132.3	24	43.0	663.3	12.3	44.0	7.8
56	6	83.0	20.7	17.1	244.5	.....	.....	.....	.....	.....	.....

* Ov. = ovary; Ovd. = oviduct; Ut. = uterus; V.P. = ventral prostate; S.V. = seminal vesicle; C.G. = coagulating gland.

TABLE 2

SUMMARY OF RESPONSE OF ORGANS IN AVERAGE PERCENTAGE CHANGE IN WEIGHT

Treatment	Aut. Age in Days	No. of Animals	Controls		Treated		Ov.*	Ovd.	Ut.	Testis	V.P.	S.V.	C.G.
			♀♀	♂♂	♀♀	♂♂							
Equine gonadotropin (10 r.u. daily for 6 days)	6	48	8	12	11	17	−15†	0		35‡	.....	.....	.....
	10	44	11	9	14	10	15‡	18	−3	38	19	75	14
	16	26	5	7	7	7	138	106	516	80	143	156	353
	26	32	10	6	9	7	123	111	344	56	75	155	116
	36	41	7	11	9	14	809	75	195	4	42	157	135
	56	14	6	....	8	....	549	36	120	.....	.....	.....	.....
Total	.....	205	47	45	58	55	.....	.....	.....	.....	.....	.....	.....
Testosterone propionate (0.1 mg. daily for 6 days)	6	26	8	6	5	7	−10	42		−2	.....	.....	.....
	10	40	7	11	9	13	−12	137	79	−11‡	10	78	50
	16	34	7	8	10	9	−12	24	106	−17	116	180	202
	26	30	9	7	8	6	−32	−31	−38	−67	71	100	26
	36	31	9	6	9	7	−22	−24	−42	−19	20	62	44
Total	.....	161	40	38	41	42	.....	.....	.....	.....	.....	.....	.....
Estradiol benzoate (1 r.u. daily for 6 days)	6	37	10	10	6	11	−48	346		8†	.....	.....	.....
	10	40	9	11	8	12	−9	177	227	−31‡	.....	−6	
	16	35	11	7	10	7	−3	55	146	−29	−68	0	−15
	26	36	7	9	11	9	−15	−18	6	−22	−5	−9	−4
	36	32	10	7	8	7	−4	−14	−37	−46	−30	−26	−14
Total	.....	180	47	44	43	46	.....	.....	.....	.....	.....	.....	.....
Grand total	.....	546	134	127	142	143	.....	.....	.....	.....	.....	.....	.....

* Abbreviations same as in Table 1.

† Analyzed statistically—not significant.

‡ Analyzed statistically—highly significant.

the equine gonadotropin was aqueous. Injections in the three groups were started at birth and at 4, 10, 20, and 30 days of age. Animals were killed about 24 hours after the last injection, at the ages of 6, 10, 16, 26, and 36 days. In addition to these animals, a small group of females was injected with equine gonadotropin for 6 days at from 50 to 56 days of age, to study the changes in reactivity of the ovary and uterus at this age.

The time of the opening of the vagina was observed, and smears were studied in some injected and control females. The size of the clitoris and penis of injected animals was observed at autopsy and compared to these organs in the normal controls.

At autopsy the ovaries, oviducts, uteri, testes, ventral prostates, coagulating glands, and seminal vesicles were dissected rapidly to avoid drying of the tissues and were weighed on a torsion balance. The two ovaries, oviducts, testes, seminal vesicles, or coagulating glands of each animal were weighed together. At 6 days the organs were so small that the oviduct and uterus were dissected and weighed together and the male accessory organs were left attached to the urethra and sectioned together; but weights were found to be unsatisfactory. All tissues were fixed in Bouin's fluid. The ovaries, oviducts, and the anterior portion of the uteri were sectioned serially at 10 μ; samples of the male organs were sectioned at 6 μ. The tissue was stained with Harris' haematoxylin. Some duplicate samples of the male accessory organs were stained with Ehrlich's haematoxylin or modified Mallory's triple stain.

Tissues from 154 animals were studied microscopically. Measurements of the diameter of testis tubules and ovarian follicles were taken in some cases. No tissues were studied in the 56-day-old females.

The mean body weights and organ weights of all injected animals at a given age in each series were compared with those of the corresponding control animals. The percentage differences in weight are given in Table 2. In some cases in which this percentage was of doubtful significance, Fisher's formula was applied, and the t-value for significance was obtained. Body weight was not affected by any of the hormones injected at any age.

NORMAL POSTNATAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

AT 6 DAYS OF AGE

At 6 days after birth the ovary is a very small organ, with a mean weight of 0.8 mg., and is in a very simple stage of development. The primordial sex cells are arranged in clusters of irregular size and shape, which occupy mainly the outer region of the gonad. These clusters are surrounded by smaller, slightly elongated epithelial cells. The oviduct is also very simple, and the epithelium is low. The uterus is undeveloped, small in diameter, and has a low epithelium and a small, simple lumen (Fig. 4).

The testis has solid tubules, which average 46 μ in diameter. They are formed of one row of small basal epithelial cells arranged around the periphery inside the basement membrane; many cells are undergoing mitosis. The center of the tubules is packed with large, light-staining cells, in which mitoses are not observed. The stroma between the tubules consists of a large number of two kinds of cells: spindle-shaped mesenchymal cells around the outside of the basement membrane of the tubule, as well as elsewhere in the intertubular spaces; and round interstitial cells, larger than the mesenchymal cells, arranged in clumps or loose in the intertubular space.

The male accessory reproductive organs are still in the anlage stage at 6 days. The ventral prostate consists of two small lobes composed of buds from the urethra. These are hollow at the proximal end but solid at the distal tip. The seminal vesicle is a short, hollow, saclike structure, coiled anteriorly, with a branching lumen and a low epithelium. The coagulating gland was not studied histologically at this age.

AT 10 DAYS OF AGE

By 10 days of age the ovary has increased somewhat in weight, although it is still very small. It is completely filled with small follicles, but around the periphery the "egg-cluster" condition typical of the 6-day ovary is still present. The larger follicles have a mean diameter of about 53 μ and in most cases consist of one layer of follicle cells. In some follicles, however, there is very active mitosis taking place, so that a second layer is already forming and in others there are several layers. Glands are just beginning to develop in the uterus at this age. They appear as very small indentations in the uterine epithelium. The vagina is open in all females with a body weight of 8 gm. or more. The vaginal smear is scanty and consists mainly of leucocytes and debris.

The testis is now over twice as heavy as it is at 6 days, but the solid tubules have not increased very much in diameter. However, a second row of basal epithelial cells is beginning to appear inside the first row, and the large cells in the center of the tubules are less numerous and their nuclei have become vacuolated. The ventral prostate is larger than it is at 6 days. The acini are larger in diameter, although they are still solid at the tip. The epithelium is low. The seminal vesicle has a moderately high pseudostratified epithelium with elongated nuclei crowded together, and many cells show mitoses. The coagulating gland has, for the most part, small, solid acini, although a few of the acini have lumina.

AT 16 DAYS OF AGE

The ovary has a mean weight of 2.1 mg., and the largest follicles are almost twice as large as they are at 10 days. These follicles occupy the center, while very small follicles are located toward the periphery of the ovary. There are also more follicles containing several layers of follicle cells than there are at the previous age, and very few have only one layer. Follicles having a multiple number of ova are very numerous at 16 days and remain quite numerous for some time after this. These polyovular follicles may contain 2–6 ova, which, in most cases, are separated from each other by rows of future granulosa cells. The epithelium of the oviduct is much higher than at 10 days. The nuclei are basal, and the cytoplasm is vacuolated. The epithelium of the uterus is now folded, and the elongated nuclei occupy most of the cell. The uterine glands have grown markedly since the last age. They are much more numerous, and some are beginning to form lumina. The vaginal smear is no longer scanty in some females at this age, and it consists of many cornified cells, leucocytes, and nucleated epithelial cells, plus some atypical material which may be classified as debris. In some cases all three cell types are present; in others, two types are found.

The testis has grown tremendously and has a mean weight of 51.3 mg. The tubules are larger in diameter than at the previous age, and some of them are beginning to acquire a small lumen. There are several rows of small epithelial cells. Many of these cells, bounding the small lumen, are dark, and their nuclei appear to be either dividing or degenerating. According to Hargitt (1926), in the rat

these are degenerating spermatogonia. This is probably the case in the hamster also, since at 26 days the number of cells in the tubule is considerably reduced. By 16 days all the large cells in the center of the tubule have disappeared. In the spaces between the tubules, the round interstitial cells appear less numerous than at 10 days of age.

The ventral prostate, although still very light in weight, is greatly developed as compared to the previous age. It has many large and highly convoluted acini. The epithelium is high and folded and so-called "light" areas are present in the cells above the basal nuclei. These light areas are very similar to the ones described in the ventral prostate of the rat (Moore, Price, and Gallagher, 1930). At this age they are still rather small and indefinite, since they are just beginning to develop. The seminal vesicle has doubled in weight since 10 days, but it does not appear much more developed. The coagulating gland is approximately the same size and has the same differentiation as before.

AT 26 DAYS OF AGE

At 26 days of age, hamsters are extremely variable in size, the body weights ranging from 22 to 52 gm. The weight of the ovary, as well as its degree of differentiation, is also variable. Ovarian weight ranges from 10 to 20.6 mg., with a mean weight of 14.6 mg. (Table 1). In general, the smaller animals have the lighter and less well-developed ovaries, although exceptions to this condition are numerous. The simpler ovaries have many rather small follicles, about 100–150 μ in diameter and without antra, while the heavier ovaries have many follicles of 200–385 μ in diameter and with antra. Polyovular follicles are abundant in all the ovaries examined in 26-day-old hamsters. In one ovary, 130 polyovular follicles were found. Most of these follicles have two ova, although some have three or four. Corpora lutea are completely absent in ovaries at this age, which shows that ovulation has not yet occurred, even in the largest animals.

The uterus is much larger than it is at 16 days and is extremely variable. Its weight ranges from 16.4 to 134 mg., and there seems to be little or no correlation between uterine size and the weight of the animal. Uterine glands are very numerous and branching. They extend through the endometrium, almost to the muscle layer. The larger uteri have a very folded mucosa and a high, pseudostratified epithelium, while the smaller uteri have a simple mucosa with a lower epithelium.

The testes have increased markedly in weight since 16 days of age and have already descended into the scrotal sac in some animals, although in others they are still in the inguinal canal. All the tubules have lumina, but the lumina are larger in the tubules of heavier testes. Tubules with the larger lumina have a very few of the small epithelial cells. There are many primary spermatocytes in the process of being exfoliated into the cavity. Interstitial tissue is very sparse.

The ventral prostate has large, distended acini, with high epithelium. The light areas are more marked than they are at 16 days. The seminal vesicle is very much larger and more highly developed. The epithelium is extremely convoluted, although perhaps lower than that of the ventral prostate. Above the nucleus there is a round or oval light spot, comparable to the light area in the ventral prostate but smaller. There is evidence of secretion in the acini. The coagulating gland is now very large and well developed. It has highly convoluted acini with high epithelium, round basal

nuclei, and clear cytoplasm. The acini seem to be filled with secretion.

AT 36 DAYS OF AGE

Hamsters have grown little in size since the previous age and are still variable in weight. The ovaries are also variable and have increased little in weight since 26 days. However, ovulation has now taken place, as evidenced by the presence of several corpora lutea in most of the ovaries studied and ova in the oviducts in a few cases. Polyovular follicles seem to be disintegrating, since they are found now in small numbers. The uterus is still variable in size. The epithelium is columnar, with vacuolated cytoplasm. The uterine glands appear to be at the same stage of development as at 26 days. The vaginal smear shows that cycles have begun in these animals.

Vaginal smears were studied in a group of nine females belonging to two litters. Examination of smears was started at the age of 24 days, and smears were taken daily for 10 days until the animals were 33 days of age. At 31 days all females had smears composed almost exclusively of a huge mass of nucleated epithelial cells. This, according to investigators (Peczenik, 1942; Kupperman, Greenblatt, and Hair, 1944; and Ward, 1946) who have studied the vaginal smear of the hamster, is the typical smear picture on the morning after ovulation has occurred.

The testes, although still variable in size, are now very much larger than they are at 26 days and have descended into the scrotal sac in all animals. The tubules are larger (100–150 μ) in diameter, and most of them have a wide lumen. They have many spermatogonia, spermatocytes, and sperm heads, although the latter are not present in the smaller testes. Interstitial cells are very sparse. The light areas in the epithelial cells of the ventral prostate and the seminal vesicle are sharper and more definite than at previous ages. Figure 3 shows these light areas in the seminal vesicle. The coagulating gland does not seem to have developed a great deal since 26 days of age.

SUMMARY OF NORMAL DEVELOPMENT

The normal development of the reproductive tract of the golden hamster can be summarized as follows: The ovary of the hamster grows very slowly during the first 16 days after birth (Table 1). Ovarian growth is suddenly accelerated between 16 and 26 days and is slow again during the next 20 days. At 6 days of age the condition of this organ is extremely primitive, and it consists of clusters of primary sex cells surrounded by epithelial cells. Follicles are formed by 10 days, some of which grow steadily and acquire antra by 26 days of age, while others degenerate. The first ovulation has occurred by 36 days, when corpora lutea are first found in the ovary.

The uterus grows much faster than the ovary, but it also has a sudden acceleration of growth after 16 days. Uterine glands first appear at 10 days, acquire lumina at 16 days, and are fully developed by 26 days of age. The vagina normally opens when the females reach a weight of about 8 gm. This usually occurs around 10 days of age. The vaginal smear is very scanty at first and contains mainly leucocytes and some debris. Cornified and nucleated epithelial cells soon appear. Around 30 days of age, cycles have started.

The testes grow very rapidly during the first 36 days of life, but the greatest increase in weight occurs between 16 and 26 days. By 26 days they are beginning to descend, and at 36 they are in the scrotal sac. At 6 days of age the testis is quite primitive in structure. It has solid

tubules with one row of basal epithelial cells. A very small lumen is beginning to appear in the tubules at 16 days; it gradually increases in size until 36 days of age, when it has reached its maximum diameter. Sperm heads appear at about 36 days. Interstitial tissue is most abundant at 6 and 10 days, after which it appears to decrease and is very sparse at 26 and 36 days.

The male accessory organs are extremely small and simple in structure during the first 2 weeks of life. There is a sudden acceleration of growth and development between 16 and 26 days. At 16 days of age the ventral prostate acquires lumina in all the acini, and light areas appear in the epithelium. These, however, are not fully developed until much later in life. The seminal vesicle acquires similar light areas in the epithelium at 26 days, but they are round or oval and smaller than in the epithelial cells of the ventral prostate. The coagulating gland has a high epithelium and round basal nuclei.

EFFECT OF HORMONE INJECTIONS

I. EQUINE GONADOTROPIN

AT 6 DAYS OF AGE

It was very difficult to accumulate enough data on animals injected from birth to 6 days of age because the mother hamster has a tendency to destroy her young if she is disturbed in any way soon after littering. Moreover, marking the treated animals by cutting off the ear or the tip of the tail was very unsatisfactory, since the bleeding was a great factor in stimulating the mother to destroy the litter. Tying the tip of the tail with fine silk proved to be a very adequate marking system, but this method was not used until the experiments were nearly completed. In an effort to avoid marking, some entire litters were injected, leaving no littermates for control animals, while other litters were killed at 6 days to serve as normal controls. This was done in the gonadotropic series in one-half of the cases in the females and in about one-third of the cases in the males. This procedure was extremely unsatisfactory, since our colony of hamsters has not been closely inbred and litters differ greatly as to body weight and weight of the reproductive organs.

At 6 days of age there is a 15 per cent reduction in weight of the ovary (Table 2), which is not statistically significant. No histological changes from the normal can be observed in the ovaries. The oviduct and uterus of the injected females are not affected in weight or histology. Therefore, it can be concluded from these results that the injection of equine gonadotropin from birth to 6 days has no detectable effect on the reproductive system of the female hamster.

The 35 per cent increase in the testis weight is statistically significant and has a t-value of 4.2, with a probability of $< .001$. This percentage increase represents a consistent increase in testis weight in every single animal injected with equine gonadotropin when it is compared with its uninjected littermates. Histological studies, however, show no difference between the testes of the injected and the uninjected males. There are no histological changes in the accessory organs of the male.

AT 10 DAYS OF AGE

Injections of the gonadotropic hormone produce an increase in the ovary of 15 per cent, with a t-value of 4.2, which represents a probability of $< .001$. The diameter of the follicles is increased from 53 to 77 μ. The oviduct weight is increased 18 per cent, and histological changes from the normal are observed. The epithelium appears greatly stimu-

lated; the cells are higher, and there are more mitoses than in the epithelium of the oviduct of the control females. Although the uterus is not increased consistently in weight, there is some evidence of histological stimulation. The epithelium seems higher, and there are more gland crypts in the uterus of the injected animal. In one particular case the uterus is exceptionally increased in weight and shows tremendous stimulation. This uterus weighs 32.4 mg., while the mean uterine weight of the injected animals is 7.94 mg. Its diameter is greatly enlarged, and the epithelium is much higher and folded. The uterine glands are larger and more numerous and with lumina beginning to form. The muscle layer is also better developed.

The testis is stimulated in its growth to about the same degree as in the 6-day-old males. As before, the testes of all the injected males are consistently heavier than those of the noninjected animals, so that, in spite of the absence of histological differences, the testes of hamsters at 10 days are definitely stimulated by this gonadotropic agent. The ventral prostate is increased 19 per cent in weight, but it shows no histological changes from the normal. The seminal vesicles are increased 75 per cent in weight. This represents a consistent increase in all injected animals. In spite of the simplicity of the seminal vesicle at this age, its lumina are larger, and the epithelium is much higher than in the control. The coagulating gland is increased in weight 14 per cent over the control, but no histological changes are observed. The penis is enlarged in some injected animals.

AT 16 DAYS OF AGE

The ovary has increased 138 per cent in weight (Table 2), and the follicular diameter and the theca seem to be slightly enlarged by the gonadotropic injection. The oviduct is increased 106 per cent over that of the normal animal, and it has a larger diameter and a greatly stimulated epithelium. The uterus reaches its maximum increase in weight over the normal at this age in the gonadotropic series, and it shows great histological stimulation. The diameter is greatly enlarged, the epithelium more folded and very much higher, appearing pseudo-stratified, and the cells are vacuolated.

The epithelium in the uterine glands is also vacuolated, the glands are greatly enlarged and have much larger lumina than the glands of the normal animal. The clitoris in some injected females is enlarged and seems swollen and wider than the clitoris in normal females.

The testis is now increased 80 per cent over the normal weight for this age. There is also an increase in tubular diameter of from 64 to 82 μ. Lumina are present in all tubules of the testes of injected males and are larger than the lumina of normal testis tubules at this age. The ventral prostate has now reached its maximum percentage increase in weight and has more acini than the normal gland. The epithelium is more folded but not any higher, and there are light areas in the gland of the normal, as well as of the injected, animals. The seminal vesicle has also reached its maximum weight increase, and the histology of this gland in the injected male shows great stimulation over that of the control. The epithelium is much higher and folded and has precocious light areas. These are pale and small as compared to the light areas of the older glands. The coagulating gland also reaches its peak of reactivity at this age, with a weight increase of 353 per cent over the normal weight. The epithelium is very much stimulated; it is folded and much higher in the gland of

the injected animal. The penis is enlarged at this age.

AT 26 DAYS OF AGE

The ovary is increased in weight to about the same degree as it is at 16 days, but it has large follicles, 230–400 μ in diameter and with antra. Although the normal littermates are of approximately the same body weight as the injected females, their ovaries have follicles below 150 μ in diameter and without antra. There is some thecal and interstitial luteinization in the ovaries of the injected females. The oviduct is enlarged 111 per cent but is not affected histologically. The uterus is increased 344 per cent over that of the control. The epithelium is more folded; the cells are tall and columnar with small, round, basal nuclei and vacuolated cytoplasm. The uterine glands are not very numerous, but more of them have lumina than the normal uterus. The muscular layer is more prominent. The epithelium of the normal uterus is lower and has elongated nuclei, and the cytoplasm of the cells is not vacuolated.

The testis is not increased so much over the normal weight as it is at 16 days; however, gonadotropic stimulation is evidenced by an increase in average tubular diameter of from 87.5 to 112 μ. There is also some indication of an increase in interstitial tissue. The ventral prostate is increased 75 per cent in weight, the seminal vesicles 155 per cent, and the coagulating gland 116 per cent. However, no histological changes are found.

AT 36 DAYS OF AGE

At this age the ovary reaches its peak of reactivity, as shown by the weight increase of 809 per cent (Table 2) and by the histological picture. The weight of the ovary is increased from a mean of 17.4 mg. in the controls to 146.4 mg. in the injected animals. Figure 1 shows the degree of stimulation of the follicles when compared with Figure 2, the ovary of a normal littermate female. Most follicles in the ovary are enlarged tremendously and have huge antra. These large follicles give the ovary a mulberry appearance when examined macroscopically. Many blood points are present; more than twelve have been observed in one ovary. Some thecal luteinization is found, but there are no corpora lutea. The oviduct is increased 75 per cent but shows no histological changes from the normal. The uterus is increased 195 per cent in weight. Its diameter is enlarged, the mucosa is much more folded, and the epithelium is higher than in the uterus of the normal animal. It is difficult to determine whether the clitoris is enlarged in females at this age.

The weight of the testis is not affected at 36 days. Interstitial tissue still appears more abundant in the testis of the injected male. The ventral prostate is increased 42 per cent in weight over that of the control but is not affected histologically. The seminal vesicle is increased in weight to about the same degree as in the last two ages. Acini are more distended than in the normal gland, and the epithelium is more folded. The coagulating gland is increased 135 per cent in weight, but its histology is not affected. The penis is enlarged in only a few animals; in most it is difficult to determine.

AT 56 DAYS OF AGE

In view of the very high reactivity of the ovary at 36 days, some females were injected daily at from 50 to 65 days of age in order to determine the ovarian sensitivity at an older age. These females show a decrease in reactivity of the ovary to gonadotropic stimulation. The percentage increase in ovarian weight of injected animals has dropped from 809 per cent

at 36 days to 549 per cent at 56 days. Although no histological study was made of the tissue at this age, macroscopic observation of the ovary shows extreme follicular growth, a great number of hemorrhagic follicles, and some corpora lutea. This gives the ovary the mulberry appearance already observed at 36 days of age. The reactivity of the oviduct and uterus is also lower than at the previous age. The weight increase of the oviduct is now 36 per cent and that of the uterus 120 per cent.

II. TESTOSTERONE PROPIONATE

AT 6 DAYS OF AGE

It was even more difficult to gather enough data on animals injected from birth to 6 days with the sex hormones than it was with equine gonadotropin because of the oily medium of the sex hormones. Even when no seepage of the oil occurred at the time of the injection, the animals acquired the odor of the oil, which was probably a disturbing factor for the mother. This caused the destruction of so many litters that the amount of data obtained is inadequate. Because of the small number of cases, the percentage weight changes were not analyzed statistically.

The ovary at this age ranges in weight from 0.4 to 1.0 mg., with a mean of 0.8 mg. in the control, and from 0.4 to 1.4 mg., with a mean of 0.72 mg., in the injected animals. There are no histological differences between the ovaries of the injected and the control animals. In the female accessory organs, however, the percentage increase in weight over the normal is very marked and is probably significant. It represents an increase of from 6.2 to 8.8 mg. This increase is quite consistent in all the injected animals and is accompanied by very marked histological changes. The oviduct has a much higher epithelium; the uterus is greatly increased in diameter, and the epithelium is folded and higher than in the uterus of the normal animal. Uterine glands are produced precociously by direct stimulation, and, although they are not very numerous, they are very conspicuous, and some are beginning to acquire lumina. This precocious development of uterine glands is very similar to that produced with estradiol benzoate (Fig. 4). The clitoris is greatly enlarged and is wider and longer than in the normal female.

The weight of the testis is not affected at this age, and there are no detectable histological differences between the testes and accessory reproductive organs of the injected and the control males. The penis is greatly enlarged.

AT 10 DAYS OF AGE

Reduction in the weight of the ovary at this age is again of doubtful significance because of the small number of cases. It represents a weight change from a mean of 1.08–0.96 mg., and there is not a consistent decrease in all the injected animals. There are no changes in the histology of the ovary of injected females at this age or at any of the subsequent ages. At 10 days the oviduct reaches its highest percentage increase in weight, which is much above that of the next age. It is greatly stimulated and shows great histological changes. The epithelium is extremely high and somewhat pseudostratified in places. The uterus is increased 79 per cent in weight over the control. The diameter is greatly enlarged, and the epithelium is more folded than in the control animal. The smooth-muscle layer is wider and appears more distinct. Uterine glands are highly stimulated; they are very large and have acquired a lumen. The vaginal smear is not affected by male hormone at this age. The clitoris is greatly enlarged.

The 11 per cent reduction in the weight of the testes is highly significant and has a *t*-value of 4.8, although reductions are not found in all the injected animals. There are no changes in the histological picture. The ventral prostate is increased only 10 per cent in weight, but the gland appears more developed and has more acini with lumina than the gland of the control male. Some acini have a high epithelium, although no light areas are produced precociously by the male hormone. The seminal vesicle is increased 78 per cent, and its epithelium is higher and more folded. The coagulating gland is also stimulated and has a weight increase of 50 per cent, but no histological differences are apparent. The penis is enlarged.

AT 16 DAYS OF AGE

The ovary is reduced in weight to the same degree as at the previous age, and its histology is not affected. The oviduct is stimulated much less at this age than it is at 10 days. Its weight increase is only 24 per cent, and the epithelium shows less indication of stimulation, although it is still higher than is the oviduct of the control females. The uterus is stimulated more at 16 days than it is at earlier ages both in weight increase and in uterine gland growth. The clitoris in some of the injected females is larger than the penis of the normal 16-day-old male.

The testis is reduced 17 per cent in weight but shows no histological changes as compared to the control. The ventral prostate is increased 116 per cent, reaching at this age its maximum percentage weight increase with male hormone. The acini are larger, but the epithelium is not higher than in the normal. Light areas are present in the prostate of both injected and control animals and are not larger or more conspicuous in the gland of the injected animal. The seminal vesicle is increased 180 per cent, which also represents a peak of reactivity. The epithelium is a little higher in the treated males; and small, precocious light areas are produced. The coagulating gland also reaches its maximum weight increase at this age. It is increased 202 per cent and appears more developed than in the untreated male. The acini are larger and more numerous, and the epithelium is higher. All injected males show a remarkable increase in the size of the penis.

AT 26 DAYS OF AGE

By 26 days the ovary is reduced 32 per cent in weight. This represents a reduction in mean weight of from 14.6 (range: 10.2–18.0 mg.) to 9.8 mg. (range: 8.2–10.8 mg.). The ovarian weight is consistently reduced in all injected animals, when compared to littermate, as well as to nonlittermate control females. There are no histological changes in the ovary. The oviduct is reduced in weight by 31 per cent, but its histology is not affected. The uterus is reduced 38 per cent. The normal uterus is so variable in weight (16.4–134.0 mg.) and in histology in the 26-day-old females that it is very difficult to determine whether this reduction is really due to the male hormone. The clitoris of injected females is as large as the penis of the normal male of the same age.

The testis is reduced 67 per cent in weight and shows inhibition of tubule growth in diameter and of lumen formation. The average diameter of the tubules is reduced from 84 to 65 μ. While most tubules in the testes of control males have either a large lumen or a small one beginning to form, there are almost no tubules with lumina in the testes of the injected males. The ventral prostate is not increased in weight as much as it is at the previous age. The acini are greatly

distended in the gland of the injected male, but the epithelium is not higher. The increase in weight of the seminal vesicle is now only 100 per cent, and the coagulating gland has an increase of only 26 per cent. There are no histological changes from the normal in either gland. The penis is still enlarged by the injection of testosterone propionate.

AT 36 DAYS OF AGE

The ovary shows a reduction of 22 per cent in weight, accompanied by no histological changes. The accessory female organs are reduced to about the same degree as in the previous age and show no detectable histological changes from the normal.

The testis is reduced 19 per cent in weight, and there are no histological differences from the normal. Sperm heads are found in the testes of both normal and injected males. The ventral prostate is increased 62 per cent and has some acini that are much more distended than in the normal gland. The seminal vesicle is not so enlarged as it is in the previous age and is not affected histologically. The coagulating gland is enlarged 44 per cent in weight and shows no histological difference from the controls. The penis is still enlarged by the injection.

III. ESTRADIOL BENZOATE

AT 6 DAYS OF AGE

There is a 45 per cent reduction in the weight of the ovary at 6 days of age. This represents a reduction in mean weight of from 0.5 to 0.26 mg. The significance of this reduction was not determined because of the small number of cases. The oviduct and uterus, however, are so tremendously increased in size in every injected female that, in spite of the few cases, it is unquestionable that these organs are really stimulated by the injection of female hormone. Table 2 shows a weight increase of 346 per cent of oviduct and uterus together. This is an increase in mean weight of from 5.1 to 22.8 mg. Histologically, the oviduct shows an extremely high epithelium, with vacuolated cytoplasm. The uterus is very much enlarged in diameter, the endometrium and the lumen are larger, and the epithelium is higher and folded. Uterine glands are precociously developed; some are in the crypt stage, but others are much larger and more developed and are beginning to acquire a lumen (see Figs. 4 and 5).

The injection of female hormone opened the vagina in all the females in this series, even though their body weight range was between 5.3 and 6.3 gm., which is very much below the weight at which the vagina normally opens in the hamster. The smear taken at 6 days of age in these injected females was very abundant and consisted of either a definite mass of nucleated epithelial and cornified cells or a combination of cornified cells, leucocytes, and epithelial cells. The clitoris is wider and longer than in the normal animals, and the whole genital region appears swollen.

There is no significant weight change in the testis of animals 6 days of age, and its histology is not affected. No histological differences are observed in any of the accessory organs of the treated, as compared to the untreated males. The penis is also unaffected.

AT 10 DAYS OF AGE

The ovary is not affected in weight or in histology. The oviduct and uterus are stimulated to about the same degree as they are at 6 days, as determined by the percentage increase of their combined weights. The uterine glands of the injected animals are now long and coiled and have large lumina. They seem to be more numerous than in the normal animal. The vagina is open, as before, in all

injected animals. The smear on the tenth day consists of a mass of cornified cells. The clitoris is enlarged and the genital region highly swollen.

The testis is reduced 31 per cent in weight, which is highly significant (t-value = 5.1). No histological changes are observed. The ventral prostate is reduced so much in size that it was impossible to dissect it from the urethra, so it was fixed *in situ*, and no weights were obtained. It does not differ histologically from the normal gland at this age. The seminal vesicles and the coagulating gland were dissected, weighed, and sectioned together. There is no significant reduction in their weights and no histological change from the normal. The penis is now reduced in size.

AT 16 DAYS OF AGE

The ovary is not affected; the oviduct is increased only 55 per cent in weight, and it is difficult to detect any histological difference between the oviduct of the injected and the control females. The uterine increase of 146 per cent is less than it is at 10 days. Histologically, however, the degree of stimulation seems about as great as it is at the previous age. The uterine glands are larger, more numerous, and have larger lumina than the glands of the normal uterus. The vaginal smear of the injected females is very abundant and consists of either a large mass of cornified cells and a few nucleated epithelial cells or a mass of both cell types in approximately equal proportions. The clitoris is enlarged, but no swelling of the genital region is observed.

The testis is reduced about the same amount in weight as it is at the previous age and shows no histological differences from the normal. The ventral prostate is reduced 68 per cent, and there is a reduction in epithelial height and an inhibition of light areas which are normally present in the ventral prostate at this age. The seminal vesicle is not affected in weight or in histology. The coagulating gland is reduced 15 per cent in weight but does not differ histologically from the normal. The penis is again reduced in size.

AT 26 DAYS OF AGE

The ovary is reduced 15 per cent in weight, the oviduct 18 per cent, while the uterus is increased 6 per cent. In view of the small number of cases, it is very likely that the reduction in the ovary and the oviduct and the slight increase in the uterus have little or no significance. This is especially true in the case of the uterus, which has already been described as extremely variable in weight at this age and at 36 days. The vaginal smear in the injected female is difficult to distinguish from that of a normal animal. It is doubtful if the clitoris is enlarged.

The testis is reduced 22 per cent, and there is some slight indication of reduction of tubular diameter and inhibition in the formation of lumina, but this is not consistent in all the treated animals. The male accessory organs and the penis are unaffected.

AT 36 DAYS OF AGE

There is no effect on the weight of the ovary, but there is some indication of inhibition of follicular growth, although it is questionable. The reduction in weight in the female accessory organs is probably merely apparent, because of the great variation in the normal organs. In addition, all normal females now have cycles, so that the effect of the injection may depend upon the stage of the cycle at which treatment is begun. The clitoris and the vaginal smear are not affected.

The testis is more reduced in weight by the injection of female hormone than at any other age, and this reduction is consistent in the testes of all the injected

males. There is again some inhibition of the growth of the lumen in the tubules, although it is still doubtful because of the inadequate number of cases. All the male accessory organs are reduced in weight, and their reduction is consistent in all cases. The penis is not affected.

In summary, the effect of hormone injection in the golden hamster during the first 36 days of life can be reviewed as follows:

Equine gonadotropin.—Gonadotropic stimulation of the ovary occurs for the first time at 10 days. At this age there is evidence from the histological study of the uterus and the oviduct that the ovary is stimulated to increase its production of female hormone. There is also a significant increase in growth of the ovary as a whole. The reactivity of the female gonad is suddenly increased to a peak at 36 days. At 56 days, reactivity is still very high but lower than at 36 days. Histologically, gonadotropic injections enlarge the size of the follicles at 10 and 16 days and the thecal layers at 16 days. At 26 days, follicular growth and formation of antra are stimulated, but the highest degree of follicular stimulation occurs at 36 days (Fig. 1). The injections do not cause the production of corpora lutea, but thecal luteinization at 26 and 36 days is obvious. The clitoris is enlarged at 16 and 26 days.

The female accessory organs are very reactive to the endogenous gonadal hormone secreted as a result of ovarian stimulation by equine gonadotropin. The oviduct responds as early as 10 days by increase in weight and epithelial height. Its percentage weight change increases until 26 days, after which it drops. Some degree of reactivity in the uterus is apparent histologically at 10 days. At 16 days a sudden peak is reached in the weight of the uterus of the injected animal, accompanied by great stimulation of the uterine glands and mucosa. The percentage increase in weight drops gradually and consistently for the next 40 days.

The testis is stimulated in its growth by equine gonadotropin as early as 6 days. After the first age there are evidences from the histological condition and increase in weight of the male accessory glands that the testis is stimulated to increase its production of male hormone. The reactivity of the gonad to this dose of gonadotropic hormone is highest at 16 days and decreases gradually to become absent by 36 days. Tubule diameter and lumen size are increased at 16 and 26 days. Interstitial tissue seems somewhat increased at 26 and 36 days.

The male accessory glands reach their maximum reactivity at 16 days, as determined by their weight increase and histological stimulation. In the ventral prostate and coagulating gland this percentage weight increase drops rather suddenly after 16 days, but in the seminal vesicle the same increase in weight is preserved in all the subsequent ages. With gonadotropic treatment the seminal vesicles acquire precocious light areas in the epithelium as early as 16 days, although these are very small and indistinct. The penis is enlarged in all injected animals after 6 days of age.

Testosterone propionate.—With this hormone, the ovary is slightly reduced in weight at all ages until 26 days, when the reduction becomes more pronounced. Testosterone propionate has no histological effect on the ovary at any age studied.

The female accessory organs are stimulated in growth in the first three ages, and after that they are apparently reduced in size. Evidence of histological stimulation is also present during the first three ages and absent in the older animals. Uterine glands are produced

precociously at 6 days by the injection of testosterone propionate and are highly stimulated at 10 and 16 days. The clitoris is greatly enlarged with male hormone at all ages.

In the male, testosterone propionate reduces the testis slightly at the younger ages and more markedly at 26 days. Histological differences occur only at this age, when there is inhibition of the formation of lumina and reduction in the diameter of the tubules.

The male accessory glands all reach their maximum reactivity to male hormone at the age of 16 days. Light areas are precociously produced in the seminal vesicle at 16 days. The penis is greatly enlarged at all ages with male hormone.

Estradiol benzoate.—In the dose used, this hormone has little or no effect on the growth and histology of the ovary at any age studied. The female accessory organs respond to this dose of female hormone with a very high degree of stimulation in growth and development at the ages of 6 and 10 days, decreasing gradually in older ages. Uterine glands are produced precociously at 6 days, and highly stimulated at 10 and 16 days. The clitoris is greatly enlarged until 26 days. The genital region is highly swollen at 6 and 10 days. The vagina is precociously opened at 6 days, and the smear of the injected animals at the younger ages is composed of cornified and epithelial cells.

The testis is reduced in weight consistently after 6 days, although no inhibition can be detected histologically. The production of male hormone is inhibited to some degree, since accessory organs are somewhat reduced in weight at certain ages, and the development of light areas in the ventral prostate at 16 days is inhibited completely and the penis reduced in size at 10 and 16 days of age.

DISCUSSION

The rapidity of development observed in the reproductive system of the hamster during embryonic life (Graves, 1945; Ortiz, 1945) is evident in the postnatal development of this system in only some respects. While differentiation occurs more rapidly in certain phases of development in some organs, in others it occurs at approximately the same speed, or even at a slower rate than in the rat or the mouse.

The hamster ovary serves as an example of a rapidly maturing organ. It is far less developed at birth than is that of the rat; but, in spite of this, ovulation occurs at 30 days of age (Graves, 1945), and a fertile copulation was obtained by Selle (1945) in a female hamster at 28 days, while in the rat ovulation has been reported at ages ranging from 40 to 70 days, depending upon the strain of rats (Long and Evans, 1922; Hargitt, 1930; Blandau and Money, 1943).

A peculiar characteristic in the early maturation of the hamster ovary is that the rapidity of development is limited to a short period just before ovulation. Follicular development is very slow, and antra are only beginning to form in some follicles at 26 days of age, as reported in the present work. This delayed formation of antra is in sharp contrast to the condition in the rat, in which follicular antra are present at 10 days (Price and Ortiz, 1944) and 11 days (Hargitt, 1930), and in the mouse, in which antra have been reported at 12–15 days (Engle, 1931). There is evidently in the hamster ovary a sudden and marked growth in some of the follicles after 26 days of age, resulting in ovulation within a few days, while the great majority of the follicles remain small and undeveloped (Fig. 2). This corroborates the findings of Deanesly (1938), who reported that antra appear just before ovulation.

The large number of polyovular follicles is a characteristic of the hamster ovary. Although these follicles have been reported in the rat (Lane, 1938), they are very rare in this animal but are quite common in the opossum, dog, cat, and some other mammals (Hartman, 1926). As in all these animals, polyovular follicles are more numerous in the ovaries at younger ages. They degenerate gradually and disappear almost completely before puberty.

The beginning of oestrous cycles in the hamster is closely associated with the first ovulation, as is the case in the rat. The first spontaneous oestrus in our hamster colony was observed around 30 days. Peczenik (1942) reports the first oestrus in his colony at 32 days of age, and Selle (1945) in one female, at 28 days. However, vaginal opening in the hamster is not a phenomenon that occurs as ovulation and oestrus cycles begin but is extremely precocious. In the normal females studied, the vagina opened at approximately 10 days of age, which coincides with the observations of Bond (1945) and Kupperman, Greenblatt, and Hair (1944). In the rat the opening of the vagina occurs around 50 days of age (Wade and Haselwood, 1941; Blandeau and Money, 1943). Therefore, while in the rat vaginal introitus can be considered as one of the signs of sexual maturity, in the hamster it is not linked with the maturity phenomena. This precocity of vaginal opening in the hamster, however, is not associated with early differentiation of the uterus, for the rate of development of the hamster and the rat uteri is about the same. Endometrial glands begin to form in the uterus of the rat (Price and Ortiz, 1944) and the hamster at 10 days of age.

In the male the rate of development of the reproductive tract of the hamster and the rat coincide more closely than in the female. The testis develops in both animals at almost the same speed. At birth and at 6 days the histological condition of the gonad is very similar, and the stages of differentiation which follow occur at approximately the same ages. According to Hargitt (1926), in the rat at 15 days the testis tubules begin to develop lumina; at 23 days testis descent begins, and at 38 days sperm heads appear for the first time. Testis tubules in the hamster begin to develop lumina at 16 days, testis descent starts at 26, and the first sperm heads are observed at 36 days of age. Since at this age sperm heads were not present in the testes of all animals studied, it is assumed that they are just beginning to develop and that spermatozoa are not mature and ready for fertilization until several days later. Bond (1945) did not obtain fertile copulations in males until 43 days of age.

The development of the ventral prostate gland takes place at about the same rate in the rat and the hamster. Light areas are found in the epithelium of the rat prostate at 12 days, and the gland reaches the adult histological condition before the third week of life (Price, 1936). In the hamster, light areas first appear at 16 days, and by 26 days the gland is very well developed histologically. The seminal vesicle, however, acquires the adult histological condition with cellular light areas in the hamster at 26 days, while, in the rat, secretion granules do not develop until 35 days of age (Price, 1936), so that the mature condition in the rat seminal vesicle is delayed as compared to the hamster.

An outstanding characteristic in the development of the reproductive system of the hamster is the sudden acceleration of growth of almost all the organs between 16 and 26 days of age (Table 1). Since this acceleration in organ growth is not accompanied by similar accelera-

tion in body growth, it seems logical to assume that there is either a sudden increase in organ reactivity to hormone stimulation or a sudden increase in production or release of gonadotropic hormone from the hypophysis in the normal animal at this age, which stimulates the gonads to increase in weight and in hormone production and thus affects the accessory organs. Although no work has yet been reported on assays of anterior lobe hormone content in the hamster, the work of Clark (1935) on implants of rat hypophyses assayed in young mice shows that there is a sharp rise in gonadotropic hormone content at between 13 and 20 days in the female rat.

A comparison of the effects of hormone injections in the hamster and the rat (Price and Ortiz, 1944) establishes two points: (1) in general, the reproductive system of the hamster is much less responsive than that of the rat to the same hormone doses, and (2) the period of highest reactivity occurs earlier in the hamster than in the rat. The percentage weight changes are greater in the rat in all organs with all three injected hormones; but the difference is even more marked with the gonadotropin. The greatest reactivity, as determined by percentage weight increase, is reached in the rat at 26 days with all organs except the testis, while in the hamster it occurs at 16 days or earlier in all except the ovary, which reaches its peak at 36 days.

The delayed reactivity of the ovary may be explained on the basis of the fact that follicular antra are not present in the hamster ovary until about 26 days of age, and gonadotropic hormones do not affect follicles to a great extent before antra are formed (Hertz and Hisaw, 1934). As soon as antra are present in the hamster ovary, the degree of reactivity to gonadotropic stimulation rises rapidly above that at the previous age and reaches the 36-day peak. Peczenik (1942) also found the hamster ovary most reactive to gonadotropic hormones at 30 days of age.

The loss of reactivity in the hamster ovary after 36 days is similar to that found in the rat ovary after 26 days (Price and Ortiz, 1944). While the ovary of the 56-day-old hamster injected with gonadotropin resembles that at 36 days, in the extreme enlargement of the follicles and their hemorrhagic condition, the organ shows less percentage increase in weight at this age over the normal than it does at 36 days, so that an actual loss in reactivity occurs.

The failure of production of corpora lutea in the ovaries at 26 and 36 days with gonadotropic treatment is another instance of lower reactivity in the hamster as compared with the rat, since the same dose of equine gonadotropin produced corpora lutea precociously in the rat ovaries at these ages (Price and Ortiz, 1944). The daily dose of 10 r.u. (20 IU) for 6 days produced thecal luteinization but was too low to stimulate follicular luteinization in the hamster ovary; Peczenik (1942) was able to produce some luteinization of the stroma and follicles in 24-day-old hamsters with daily doses of 200 IU of chorionic gonadotropin given for 12 days, and the formation of massive corpora lutea with anterior pituitary extract. Similar findings have been reported in the immature guinea pig by Leathem and Starkey (1940). They obtained with 5 r.u. of PMS the same results in the guinea-pig ovary that were obtained in the hamster in the present work—great follicular stimulation, no corpora lutea, and some thecal luteinization. However, with higher doses or longer periods of injection, corpora lutea were produced.

The increase in production of female hormone in the ovaries under gonado-

tropic stimulation, as judged by changes in weight and histology of the uterus and oviduct, begins at 10 days in the hamster, as in the rat (Price and Ortiz, 1944), and is evident at all the ages thereafter. Androgenic hormone is also produced under gonadotropic stimulation by 16 days of age, since the clitoris was enlarged in the injected females. This was previously reported in the hamster by Peczenik (1942) and in the rat by several investigators (Bradbury and Gaensbauer, 1939; Marx and Bradbury, 1940; Price and Ortiz, 1944; and others).

Injection of testosterone propionate and estradiol benzoate produces some inhibition of growth in the ovaries at all ages, which is slightly accentuated at 26 days. However, the doses of sex hormones used in the present experiments do not produce any detectable histologidol changes, while in the rat the same cases caused inhibition or stimulation of follicular growth, depending upon the age (Price and Ortiz, 1944). However, when the hamster ovary was subjected to chronic extrinization with pellets of diethylstilbestrol for $3\frac{1}{2}$–$8\frac{1}{2}$ months, beginning at 40 days, it was completely suppressed in its activity and consisted only of interstitial cells and a few small follicles (Koneff, Simpson, and Evans, 1946).

The oviduct and uterus are very responsive to gonadal hormone produced by injection of gonadotropin and to estradiol benzoate and less reactive to testosterone propionate, as can be seen from the percentage changes in weight. This was also true in the rat (Price and Ortiz, 1944). One of the most outstanding characteristics of the female accessory reproductive organs is that they show their highest reactivity to female hormone, as judged by weight increase, at 6 and 10 days of age. Percentage weight increase declines gradually as the animal grows older. This is not the case in the rat, in which the responsiveness of the oviduct and uterus increases gradually, reaches its peak at 26 days, and drops thereafter (Price and Ortiz, 1944).

The oviduct and uterus at 26 and 36 days are still reactive to female hormone produced by gonadotropin-stimulated ovaries, but they seem to lose their reactivity to injected female and male hormones and are even apparently reduced in weight by these hormones. Since their capacity to respond to increased amounts of female hormone is still present at these ages, as can be seen from their percentage weight increase with gonadotropic treatment, it can be assumed that the doses of sex hormones are too low to stimulate these organs at these ages. Therefore, the apparent reduction of the uterus and oviduct at 26 and 36 days with exogenous sex hormones should be interpreted as a lack of stimulation of organs which are extremely variable in size rather than as true inhibition of growth. Larger doses would probably continue to enlarge the uterus, since the implantation of pellets of diethylstilbestrol for $3\frac{1}{2}$–$8\frac{1}{2}$ months, starting at 40 days of age, increased the uterine weight in the hamster two to four times over that of the control (Koneff, Simpson, and Evans, 1946).

The early development of great reactivity of the uterus to female hormone treatment as demonstrated by the percentage weight increase is shown also in the histological differentiation. Glands are produced precociously at 6 days of age by estradiol benzoate and stimulated at 10 and 16 days. Although a maximum weight increase is not obtained with testosterone propionate, the uterus is very sensitive to the androgen in the younger ages, as shown by precocious production of glands at 6 days, which are as well de-

veloped as in estrogen-treated females. At 10 and 16 days, glands are stimulated to the same degree by all three substances. In these respects the hamster is more responsive than the rat, for the same doses of sex hormones did not stimulate the formation of uterine glands precociously in the rat. Precocious formation of uterine glands by both androgen and estrogen treatment has been reported in the opossum by Moore and Failor (1946).

The vaginal membrane of the hamster is more sensitive to estrogen than is that of the rat, since with the same dose of estradiol benzoate the vagina of the rat did not open until 18 days (Price and Ortiz, 1944), while in the hamster it opens at 6 days of age. Large doses of estrogen in the rat open the vagina at 9 days and produce an oestrous smear (Weichert and Kerrigan, 1942). The vaginal epithelium of the hamster responds to estrogen with a smear typical of either pro-oestrus or oestrus, according to the classification of Kupperman, Greenblatt, and Hair (1944), Kent and Mixner (1945), Kent and Smith (1945), and Ward (1946).

As has already been reported by Peczenik (1942) and Bruner and Witschi (1946), the hamster clitoris is very reactive to injected androgen and estrogen. This has been found in the rat by many investigators (Turner and Burkhardt, 1939; Selye, 1940; Weichert and Kerrigan, 1942; Price and Ortiz, 1944; and others).

The reactivity of the male organs of the hamster is less than that of the rat. The weight changes produced by the hormone injections are lower, and the histological changes, in general, are less pronounced in the hamster. As in the rat (Price and Ortiz, 1944), the testis is stimulated by gonadotropin at 6 days, which is earlier than the ovary in both animals. Although no signs of increased hormone production are detectable until 10 days in the hamster, testis growth is significantly stimulated at the first two ages and reaches its peak of reactivity at 16 days. The highest testis response in the rat is at 14 days and is higher than in the hamster. At 26 days the hamster testis is much less reactive, and by 36 days it seems to have lost its reactivity to this dose of equine gonadotropin.

The injection of male hormone inhibits the growth of the testis in all animals of 10 days or older but is most effective at 26 days. As has already been discussed, the testis has an increase in growth rate between the ages of 16 and 26 days; this seems to be also the period of greatest inhibition of testis growth and development by testosterone propionate.

Estradiol benzoate also inhibits the growth of the testis at all ages, beginning at 10 days, and inhibits the production of male hormone in animals 16 days and older, as is evidenced by weight reduction and histological changes of the male accessory organs at some ages, as well as by the reduction in the penis size. The dose given in these experiments, however, was not high enough to destroy the germinal epithelium or the interstitial tissue, but such destruction has been produced with higher doses and much longer periods of treatment (Koneff Simpson, and Evans, 1946).

The male accessory glands reach their peak of reactivity at 16 days with both gonadotropin and testosterone propionate injections. In the rat the seminal vesicle and coagulating gland are more reactive to male hormone at 26 days, as they approach puberty (Price and Ortiz, 1944); but in the mouse the maximum of stimulation of the seminal vesicle after treatment with gonadogen

was reached at 10 and 15 days of age (Bishop and Leathem, 1946).

SUMMARY

The normal development of the reproductive system of the golden hamster and the effects of hormones on this system were studied in 546 animals from birth to puberty. Results can be summarized as follows:

NORMAL DEVELOPMENT

1. The gonads and accessory reproductive organs in both sexes grow and develop slowly during the first 2 weeks after birth, but they have a sudden acceleration of growth at between 16 and 26 days.

2. Follicular antra first appear in the ovary at 26 days, the first spontaneous oestrus occurs around 30 days, and corpora lutea are present in the ovary for the first time by 36 days. The vagina opens as soon as the female reaches a body weight of approximately 8 gm., which is usually around 10 days of age.

3. The testis tubules begin to form lumina at 16 days, testes begin to descend at about 26 days, and sperm heads first appear by 36 days. The ventral prostate acquires light areas at 16 days, and the seminal vesicle at 26 days. By 26 days all the male accessory glands have reached their adult histological condition, and there is evidence of secretion in the seminal vesicle and coagulating gland.

EFFECT OF HORMONE INJECTIONS

Ten r.u. of equine gonadotropin, 0.1 mg. of testosterone propionate, and 1 r.u. of estradiol benzoate were injected subcutaneously for a period of 6 days, starting at birth and at 4, 10, 20, and 30 days of age. Animals were killed and autopsied about 24 hours after the last injection, at the ages of 6, 10, 16, 26, and 36 days. The reactivity of the ovary, oviduct, uterus, testis, ventral prostate, seminal vesicle, and coagulating gland was determined by the changes in weight and histology as compared to the organs of the normal controls.

1. The ovary is first stimulated by gonadotropin at 10 days, when both weight and hormone production are increased. The reactivity of the ovary to gonadotropin increases at the next ages and reaches a sudden peak at 36 days, when there is great follicular stimulation and some thecal and interstitial luteinization but no formation of corpora lutea. Testosterone propionate reduces the size of the ovary, and estradiol benzoate has little or no effect on ovarian weight. The histology of the ovary is not affected by either sex hormone.

2. The oviduct and uterus are highly stimulated by ovarian hormone, with gonadotropic treatment beginning at 10 days, but reactivity gradually decreases after 16 days. They are also stimulated by testosterone propionate up to 16 days and not affected at older ages. Estradiol benzoate produces the greatest response of these organs at 6 and 10 days and has no effect after 16 days. Uterine glands are produced precociously at 6 days by testosterone propionate and estradiol benzoate and are highly stimulated in their development at 10 and 16 days with gonadotropic treatment and the injected sex hormones.

3. The vagina is opened precociously at 6 days with estradiol benzoate and an oestrous or pro-oestrous smear is produced.

4. The testis is stimulated in growth with gonadotropin at all ages. Beginning at 10 days, there are evidences from the increase in weight and changes in histological condition of the male accessory glands that the testis is producing an in-

creased amount of male hormone under the gonadotropic treatment. The reactivity of the gonad to this dose of equine gonadotropin is highest at 16 days, decreases gradually thereafter, and is absent at 36 days. Interstitial tissue is slightly increased at 26 and 36 days. Testosterone propionate inhibits the testis slightly at the younger ages and more markedly at 26 days. Estradiol benzoate reduces the weight and the male hormone production in the testis slightly.

5. The ventral prostate, seminal vesicle, and coagulating gland are stimulated by gonadotropic treatment and by testosterone propionate and reach their peak of reactivity at 16 days. Light areas in the seminal vesicle are produced precociously at 16 days with gonadal hormone and testosterone propionate. Estradiol benzoate reduces the growth of the male accessory glands at some ages and inhibits the light areas in the prostate at 16 days.

CONCLUSIONS

1. The hamster reproductive system does not continue its rapid prenatal growth in postnatal life except in the early maturation of the ovary, the establishment of the oestrous cycles, and the extremely precocious opening of the vagina.

2. The reproductive system of the hamster is, in general, less reactive than that of the rat to the same hormone doses.

3. The age of highest reactivity of the male organs is 16 days, of the ovary 36 days, and of the female accessory organs 6 and 10 days.

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PLATE I

FIG. 1.—Ovary of a 36-day-old hamster injected with equine gonadotropin. Compare with Fig. 2 and note the stimulation of the follicles. ×30.

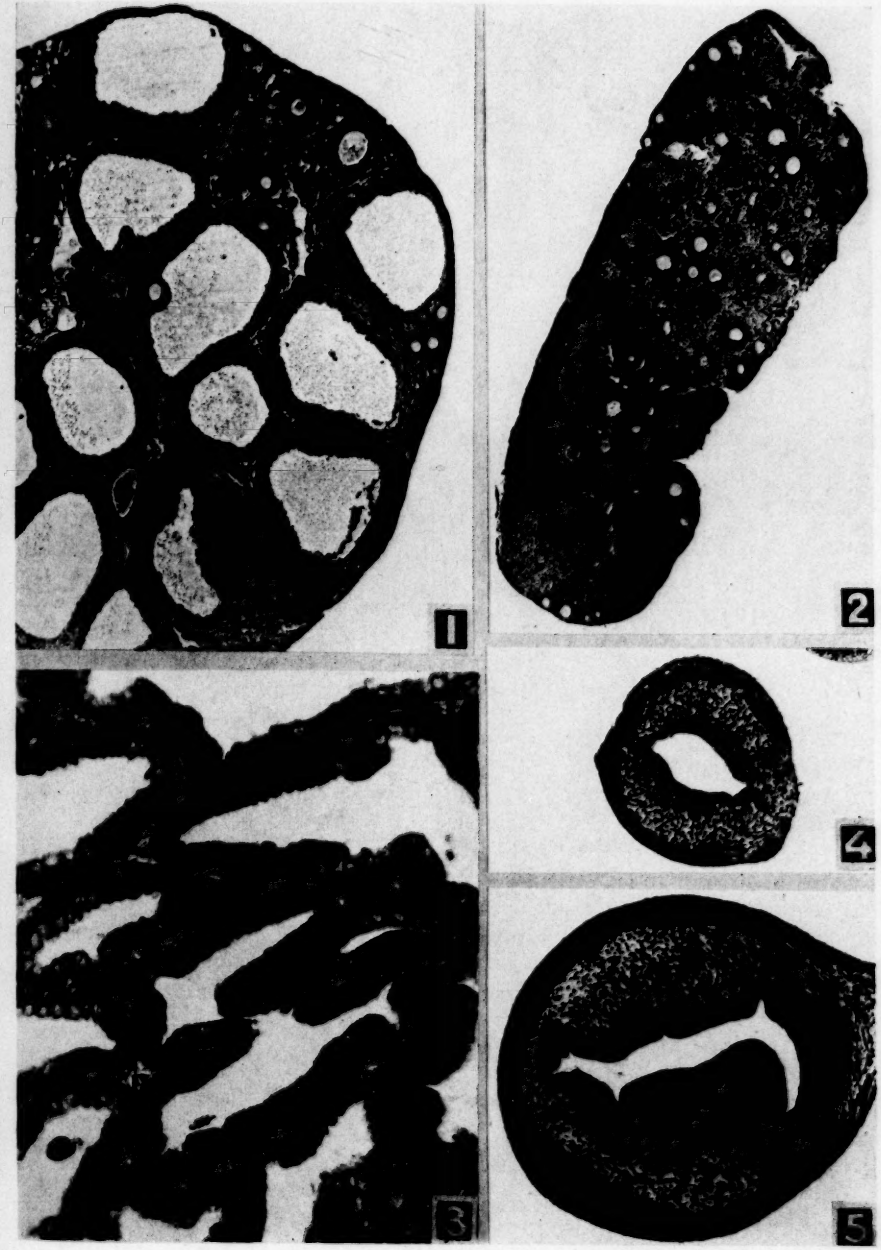
FIG. 2.—Ovary of a normal 36-day-old hamster, littermate to female of Fig. 1. Note corpus luteum forming at upper right. ×30.

FIG. 3.—Seminal vesicle of a normal 36-day-old hamster, showing light areas. ×340.

FIG. 4.—Uterus of a normal 6-day-old hamster. ×27.

FIG. 5.—Uterus of a 6-day-old hamster injected with estradiol benzoate. Compare with Fig. 4 and note stimulation of mucosa and endometrium and precocious gland development. ×27.

PLATE I



INTERRELATIONS OF THYROID AND GONAD IN THE DEVELOPMENT OF PLUMAGE AND OTHER SEX CHARACTERS IN BROWN LEGHORN ROOSTERS[1]

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I. Introduction 68

II. Materials and methods 69

III. Experimental observations 70
A. Body growth 70
B. Reproductive system 72
C. Head furnishings 75
1. Thyroidectomized roosters 75
2. Hormone therapy 76
D. Spurs 78
E. Behavior 78
F. Feather patterns 79
1. Normal male 79
2. Thyroidectomized male 79
3. Hormone therapy 83
G. Feather-growth rates 87
1. In normal fowl 87
2. In thyroidectomized roosters 88
3. Hormone therapy 89

IV. Discussion 94

V. Summary and conclusions 102

Literature cited 103

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I. Introduction

The studies of Professor F. R. Lillie and his associates (Lillie, 1942) and those of other investigators (Goodale, 1916; Benoit, 1923*a*, *b;* M. M. Zawadowsky, 1926) have demonstrated the influence of estrogens on the determination of feather color and form. Domm (1927) showed that the plumage of the Brown Leghorn hen became cocky following sinistral ovariectomy but reverted to the henny type after hypertrophy of the rudimentary right gonad. It was postulated that the reversion to henny plumage was due to secretion of estrogenic hormone by the hypertrophied right gonad; and Gray (1930), who made a careful study of the histology of these gonads in Domm's series, found evidence to support this assumption. Furthermore, removal of the hypertrophied right gonad in such hen-feathered poulards brought about a reversion to cocky plumage, which was permanently retained (Domm, 1927, 1929*a*). Additional evidence in support of the view that estrogenic hormones control the development of henny plumage in the Brown Leghorn was obtained when it was shown that the administration of these substances in roosters, capons, or bilaterally ovariectomized poulards brought about the development of normal female plumage in such individuals (Domm and Gustavson, 1929; Juhn and Gustavson, 1929, 1930; Lillie and Juhn, 1932).

The development of female or female-like plumage has also been reported in males of sexually dimorphic breeds following thyroid feeding (Cole and Reid, 1924; Torrey and Horning, 1925*a*, *b;* and others; see Schneider, 1939, for an extensive review of the early literature). With thyroid feeding, there was an extension of barbulation in the normally hackled feather areas, an increased deposition of black melanin, and a shortening and rounding of the feathers. Some investigators have designated these plumage modifications as "feminization" (Torrey and Horning, 1922; Giacomini, 1927), while others have argued the contrary (Greenwood and Blyth, 1929; Zawadowsky and Rochlin, 1928). The lesser amount of black melanin normally present or produced by thyroid feeding in the Brown Leghorn hen, as compared to the rooster, was believed by Torrey and Horning (1925*a*) to result from the secretion of an ovarian substance which nullified the action of the thyroid.

The feeding of large doses of fresh (Giacomini and Taibell, 1927) or desiccated thyroid (B. M. Zawadowsky, 1925*a*, *b;* and others) to various breeds of fowl resulted in the blanching of feathers and the development of an abnormal feather structure. These feathers were described as "silky and exhibiting a lack of hooks on the barbs" (Martin, 1929).

In cases of hypothryoid anomalies, the feathers are reported to have shown increased hackling and pigment modifications typical of the cocky plumage in a Brown Leghorn hen (Krizenecky, 1927), and a Rhode Island Red pullet (Landauer, 1929), and henny feathering in a Silver Dorking male (Buchanan, 1926).

Development of a "male-like" plumage pattern, due mainly to a decrease in barbulation, accompanied by an increased deposition of reddish-brown pigment, has been reported following thyroidectomy in the adult Brown Leghorn hen (Greenwood and Blyth, 1929; Parkes and Selye, 1937; Greenwood and Chu, 1939). Similar modifications were observed in chick, juvenile, and adult plumages when female Brown Leghorn chicks were thyroidectomized before 10 days of age (Blivaiss and Domm, 1942*b*). Thyroid secretions have been suggested to have a parallel or complementing function to theelin in determination of the

sexually dimorphic plumage pattern (Fraps and Burrows, 1939).

Following thyroidectomy in the Brown Leghorn rooster, Greenwood and Blyth (1929) and Parkes and Selye (1937) reported that some of the plumage became reddish-brown in color and lacy in appearance. The feathers of the breast, cape, saddle, wing bow, and covert exhibited a replacement of the black, red, and yellow melanins, in varying amounts by a reddish-brown pigment, accompanied by a restriction of barbulation. These modifications were not permanent, since normal plumage regenerated after the molt. Similar plumage modifications have been observed following hypophysectomy (J. B. Mitchell, 1929; Hill and Parkes, 1935*a;* Nalbandov and Card, 1943); and Hill and Parkes (1935*b*) reported a restoration to normal in such plumage following thyroxin administration.

In some fowl, thyroid administration in physiological doses was reported to result in an acceleration of gonad activity (Crew, 1925; and others). An inhibitory effect on the endocrine and gametogenic activity of the testis has been reported after thyroidectomy in the White Leghorn (Benoit and Aron, 1934) and in the Brown Leghorn (Greenwood and Chu, 1939).

The limited and often conflicting data available as regards the influence of the thyroid gland in determination of plumage pattern and gonad function in the domestic fowl suggested the need for a further systematic investigation of this problem on a larger number of birds and with a longer period of observation. This study has been concerned with the effect of thyroid removal in young chicks on the immature and mature plumage patterns and on gonad function. The form, color, and growth rate of feathers were studied in thyroidectomized birds and after treatment with thyroxin and estrogen. The gametogenic and endocrine activity of the gonads was observed, the former by histological study and the latter by observations on modifications in secondary sex characters and behavior before and after treatment with thyroxin, estrogens, and androgens. Questions considered are: (1) What are the relations of the thyroid to body growth and gonad function? (2) What are the characteristics of plumage pattern and growth rate in the absence of the thyroid? (3) What are the functions of the gonadal secretions in the determination of plumage pattern and growth rate in the absence of the thyroid? (4) Are thyroid and gonad secretions necessary for expression of the normal plumage pattern and feather growth rate?

This report is based on data obtained from 98 completely thyroidectomized and 154 incompletely thyroidectomized roosters, operated on or before 10 days of age. Over 50 per cent of these were observed beyond 6 months postoperatively and some until they were 1–2 years old (see Table 1).

II. Materials and Methods

The single comb, light brown Leghorn was selected for this investigation because of its extensive use in this and other laboratories for studies on the influence of gonadal and other hormones on the physiology and morphogenesis of growing feathers.

Previous workers, as well as this author, experienced considerable difficulty in obtaining complete thyroidectomy by operation of birds beyond 1 month of age. This was believed to result from the leaving of rests by the developing thyroid along its posterior migratory path (Lillie, 1936). These rests may hypertrophy after removal of the main mass. Thyroidectomy on or before the tenth

day of age was found to be most satisfactory in the production of a complete athyroidic condition. In an effort to alleviate operative shock, removal of the two lobes was performed in separate operations between 6 and 10 days of age.

A description of the anatomy of the thyroid gland area and the procedure for thyroidectomy are omitted, since the essentials have previously been described by Greenwood and Blyth (1929), Schwarz (1930), Greenwood and Chu (1939), and Marvin and Smith (1943). Thyroidectomy was performed on over three hundred birds. The complete removal of the gland was confirmed by macroscopic examination of the thyroid area and the histological examination of all questionable tissue. Further confirmation was usually obtained by scrutinizing plumage modifications.

In order to remove any regenerating thyroid rests in operated birds, biopsies were performed on all questionable cases at intervals of 2–3 months. The number of athyroidic cases was increased by this practice. It was found necessary to keep the thyroidectomized birds at a higher room temperature (75°–80° F.) than the normals because of their inability to adjust themselves to lower temperatures.

All birds were weighed and combs measured at the time of the operation and at monthly intervals thereafter. When definitely formed feathers appeared, samples of each plumage stage were taken at regular intervals from the various body areas.

For the study of feather growth rates, a modification of the method developed by Juhn, Faulkner, and Gustavson (1931) was employed. Growth rates were studied in the region of the anterior breast, posterior breast, back, and saddle. Twenty-five papillae in five rows of five papillae each were selected and marked in comparable areas of each of the four feather regions measured. All measurements were made on birds which were at least 6 months of age, at which time the controls were sexually mature. Measurements were made at 2- or 4-day intervals.

Differences between feather growth rates of normal and thyroidectomized birds were examined for statistical significance by making use of comparisons between appropriate groups. The differences were analyzed by "Student's" *t*-test, and the results are here expressed in terms of *P* ("Student," 1925). Values of *P* less than 0.05 are customarily considered significant, whereas values less than 0.01 are very significant.

Testes for histological study were fixed in Bouin's fluid and stained with Harris haematoxylin. The adrenal and pituitary glands have been preserved for further examination.

III. Experimental Observations

A. Body Growth

To understand and interpret better the effects of thyroidectomy on the reproductive system, plumage pattern, and feather growth rate, it seemed advisable to study modifications in body growth. Varying degrees of retardation in body growth have been reported following thyroidectomy in the chicken (Greenwood and Blyth, 1929; Schwarz, 1930; Payne, 1944) and in the duck (Benoit and Aron, 1934). Since thyroidectomy is associated with a hypogonadic condition, one might expect that body growth of such individuals would be similar to that of the capon.

Completely thyroidectomized roosters have a squat, dwarflike appearance when compared with the more erect and larger normal cock (Figs. 1–3). Incompletely thyroidectomized roosters assumed a similar posture but to a lesser extent, de-

pending on the degree of hypothyroidism (Fig. 3).

The hypothyroidic birds at all ages exhibited an unusual subcutaneous obesity in the neck, back, and breast areas and internally about the cardiac, intestinal, gizzard, and rectal areas. The increased obesity probably accounts for a significant proportion of the weight gain in the thyroidectomized roosters.

The average body weight at 6 months of age of a group of seven thyroidectomized roosters, which lived to be at least 1 year old, was 736.6 gm. compared to 1,564.8 gm. for a similar group of nine controls as shown in Figure 21.

The growth curves of normal and thyroidectomized roosters showed an increasing divergence with increasing age. The percentage ratio of thyroidectomized

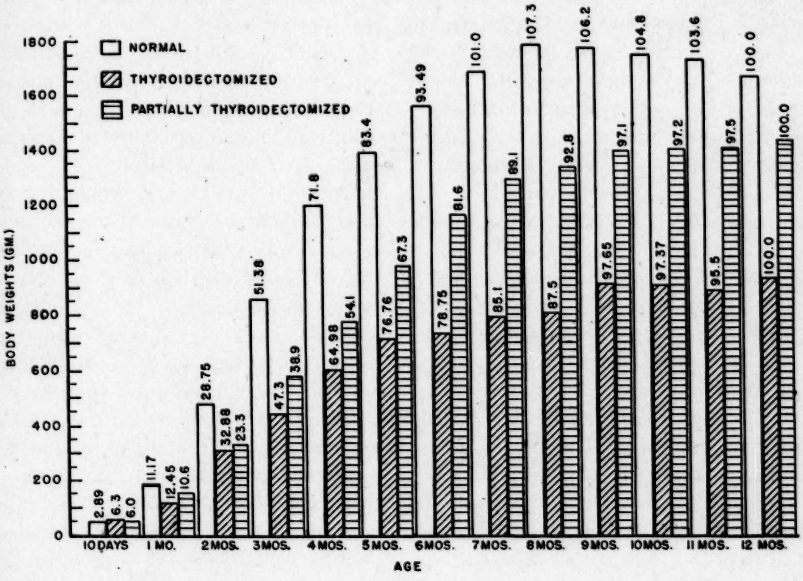

FIG. 21.—Body weights of normal and thyroidectomized roosters. Figures at top of each column are percentages of weight at 12 months of age attained at end of each month.

The skeleton, particularly the main wing and leg bones, and the viscera exhibited a reduction in size compared to controls, which was more conspicuous in the older birds than in the younger ones. The excessive length of the upper beak and of the claws observed in the athyroidic birds is no doubt a result of their infrequent use.

Completely thyroidectomized roosters exhibited a sharp decrease in growth rate, as determined by body weight, when compared with normals of the same age. to normal body weights decreased from 62.3 per cent at 1 month of age to 47.0 per cent at 6 months. The increase in this ratio to 55.8 per cent at 12 months of age resulted from the continued growth of the thyroidectomized males, while the controls reached their maximum at 6–7 months of age.

The smaller monthly increment in actual grams as well as percentage increase in body weight of the thyroidectomized rooster was not conspicuous in the first 6 months when they were only 78.75 per

cent of their 12 months' weight, compared to 93.5 per cent for the normals (Fig. 21). The athyroidic males did not reach 97 per cent of their 12 months' weight until they were 9 months of age. These data differ from those of Greenwood and Blyth[3] (1929) as regards absolute body weight and the character of the growth curve. They reported a 300–400 gm. higher absolute body weight for their adult thyroidectomized roosters than was found here at a comparable age, and a body-weight growth curve similar to that of their normals. These differences may be due to incomplete removal of the thyroid and/or the greater maturity of their birds at thyroidectomy.

The average body weight and growth curve of a group of eleven incompletely thyroidectomized roosters which lived to be at least 1 year old showed an increasing divergence from the complete operates with increasing age, as seen in Figure 21. Though the body weights of incomplete operates were greater than those of complete operates, they rarely reached the magnitude of the controls. The body weight of the incomplete operates changed from 129.7 per cent of the complete operates and 65.6 per cent of the normals at 4 months of age to 155.9 per cent of the complete operates and 87.1 per cent of the normals at 12 months of age. The increase in the percentage ratio of body weight of incomplete operates to normals with increasing age indicates the closer approach of the former to the normal absolute size and body-weight growth curve at maturity.

B. REPRODUCTIVE SYSTEM

A reduction in growth of the reproductive system and of gonad activity has been reported following thyroidectomy in mammals (Smelser, 1934; Zalesky and Wells, 1937). The reduced growth of head furnishings observed in thyroidectomized roosters (Figs. 1–3) suggests an inhibition of gonad activity, and this is confirmed by data from autopsies of such birds. Benoit and Aron (1934) and Greenwood and Chu (1939) reported a reduction in testis size and an absence of spermatozoa in thyroidectomized roosters for limited periods.

Macroscopic examination of the reproductive system of ninety-eight completely thyroidectomized roosters from 1 to 22 months of age revealed a conspicuous reduction in gross size and degree of maturity as compared to normals of the same age (Table 1 and Fig. 22). At 6 months of age, when normal roosters have reached maturity, the average weight of the testes in a group of twelve thyroidectomized roosters was 0.30 gm., compared to 14.3 gm. for a group of five controls. The testes of athyroidic roosters from 2 to 7 months of age showed a 67.8 per cent gain in weight, compared to a 98.3 per cent gain in the normals. The average size of the testes in thyroidectomized roosters at 12–22 months was not significantly greater than at 6–7 months. The reduction in testis size observed by Greenwood and Chu (1939) was less than that observed in these experiments.

In adult completely thyroidectomized roosters, the vas deferens was thin and straight, and semen could rarely be expressed from it. In normals, at 18–20 weeks of age the ductus deferens is coiled and shows an increasing diameter due to the accumulation of sperm-filled semen. The ductus deferens of thyroidectomized roosters remained relatively thin and exhibited only slight coiling even as late as 18–22 months of age. The reproductive system of these older athyroidic roosters had the appearance typical of a 2- to 3-months-old control. No other investi-

[3] This is the only previous study to provide sufficient data for comparison.

gators are known to have made any observations on the appearance of the ductus deferens in such individuals.

The lack of coiling and increase in diameter of the vas deferens of athyroidic roosters may be related to a reduction or absence of semen production. However, since growth of the vas deferens is dependent on testis hormone (Domm, 1939), its reduced size may also result from the decreased secretion and utilization of androgens.

Microscopic examination of the testes of athyroidic roosters revealed no significant histological modifications until they were 18–20 weeks old, whereas controls showed signs of spermatogenetic activity at 9–11 weeks (Figs. 4–6). The testes of normal males began to develop secondary spermatocytes at 14–18 weeks and spermatozoa at 18–20 weeks of age. The thyroidectomized males exhibited secondary spermatocytes at 22–24 weeks but did not show any spermatozoa even as late as 12–22 months of age. The tubular epithelium of the testes of thyroidectomized roosters remained relatively "cuboidal," in contrast to its more "columnar" appearance in the normal. The mitotic activity of these cells was sharply decreased in thyroidectomized males, as compared to normals at all ages. The testes of the former had a more compact appearance and a smaller seminiferous tubule and lumen than controls, and in many cases the tubules had practically no lumen. This condition was also noted by Greenwood and Chu (1939). The interstitial cells were more conspicuous in the testes of thyroidectomized

TABLE 1

TESTIS WEIGHTS OF NORMAL AND THYROIDECTOMIZED ROOSTERS

Age (Months)	Complete Thyroidectomy			Incomplete Thyroidectomy			Normal		
	No. of Cases Autopsied	Testes		No. of Cases Autopsied	Testes		No. of Cases Autopsied	Testes	
		No. of Cases	Gm.		No. of Cases	Gm.		No. of Cases	Gm.
1	8	.....	.....	5	.....	.....	.....	.....	.....
2	12	2	0.16	18	.....	.....	6	6	0.2
3	8	5	0.15	16	.....	.....	6	6	2.6
4	15	11	0.19	19	8	0.3	5	5	4.9
5	5	3	0.09	14	4	1.2	5	5	8.2
6	13	12	0.30	10	5	3.1	5	5	14.3
7	10	5	0.49	21	7	5.8	5	5	14.4
8	8	3	0.44	13	6	8.5	5	5	10.1
9	4	1	0.53	11	2	13.5	.....	.....	.....
10	6	1	0.30	9	5	9.7	5	5	13.2
11	2	1	0.24	3	2	2.9	.....	.....	.....
12	3	.....	.....	8	2	14.2	5	5	15.5
13	.....	.....	.....	1	.....	.....	.....	.....	.....
14	.....	.....	.....	3	.....	.....	.....	.....	.....
15	.....	.....	.....	1	.....	.....	.....	.....	.....
17	1	.....	.....	1	.....	.....	.....	.....	.....
18	1	.....	.....	.....	.....	.....	.....	.....	.....
19	.....	.....	.....	1	.....	.....	.....	.....	.....
20	1	.....	.....	.....	.....	.....	.....	.....	.....
22	1	.....	.....	.....	.....	.....	.....	.....	.....
Total	98	44	.....	154	41	.....	47	47	.....

roosters than in normals, which may be due in part to the smaller size of the tubules in the former. In this respect they resembled the normal during a period of its early development.

The incompletely thyroidectomized roosters exhibited varying degrees of increased testicular development when compared with completely thyroidectomized individuals. The testes of such roosters were larger than those of complete operates, though generally much smaller than normal, as shown in Table 1. The testes generally revealed a delay in spermatogenetic activity, as compared to controls, but a sharp increase over the complete operates. Some incomplete operates showed secondary spermatocytes at 14–16 weeks of age and spermatozoa at 23–25 weeks of age, which is within the normal range. However, some of these did not develop spermatozoa until 10–12 months of age. The marked delay in sexual maturation of incomplete operates

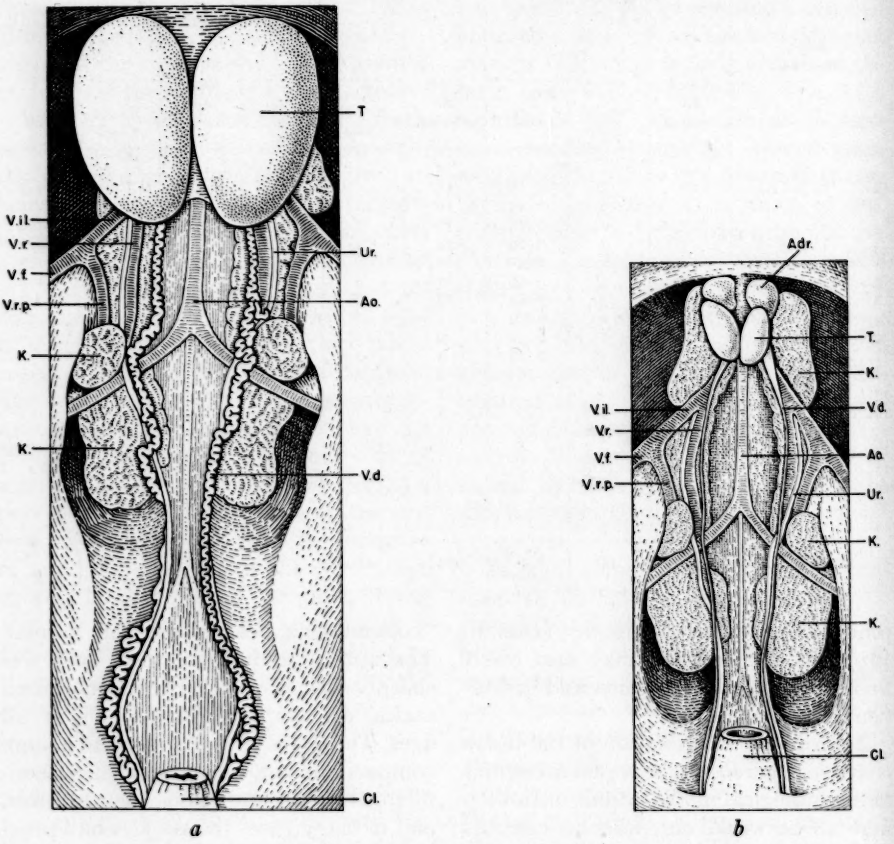

FIG. 22.—*a*, normal rooster; *b*, thyroidectomized rooster. Drawings of primary and accessory organs of reproduction of normal and thyroidectomized roosters at 7 months of age. *Adr.*, adrenal; *Ao.*, aorta; *Cl.*, cloaca; *K.*, kidney; *T.*, testes; *Ur.*, ureter; *V.d.*, vas deferens; *V.f.*, femoral vein; *V.il.*, iliac vein; *V.r.*, renal vein; *V.r.p.*, renal portal vein. Drawn to scale.

indicates that complete thyroidectomy is not necessary for inhibition of spermatogenesis or testis growth.

The ductus deferens of incompletely thyroidectomized roosters exhibited varying degrees of development from that typical of athyroidic individuals to one similar to normal. With increasing age, especially beginning at 5–6 months, the ductus deferens of incomplete operates showed an increase in diameter and coiling due to semen accumulation. However, in some, the vas deferens remained relatively straight and of small diameter, typical of the juvenile condition, until 9–12 months of age.

C. HEAD FURNISHINGS

Comb growth is known to be controlled by testis secretions, as indicated by its atrophy following gonadectomy and its hypertrophy after androgen administration. Since the testes revealed an inhibition of growth in thyroidectomized roosters, we were interested in determining to what extent this condition modified growth of the comb. A reduced growth rate and a subnormal adult size have been reported for the comb of cocks and pullets for a period of 1–4 months after thyroidectomy (Schwarz, 1930; Benoit and Aron, 1934; Greenwood and Chu, 1939; Payne, 1944). However, no data are available for size or growth curve of the comb in adult athyroidic roosters.

1. THYROIDECTOMIZED ROOSTERS

The head furnishings (comb and wattles) of normal roosters become markedly enlarged and red and turgid with development of the testes. While the head furnishings of thyroidectomized roosters in these experiments exhibited an increase in size and vascularity with increasing age, they were, nevertheless, considerably smaller than normal at all ages observed (Figs. 1–3). At 4 months of age the average comb size of a group of seven thyroidectomized males, which lived to be at least 1 year old, was 27.4[4] per cent of the comb size of a similar group of nine controls but was reduced to 18.3 per cent of the controls at 12 months, as seen in Figure 23.

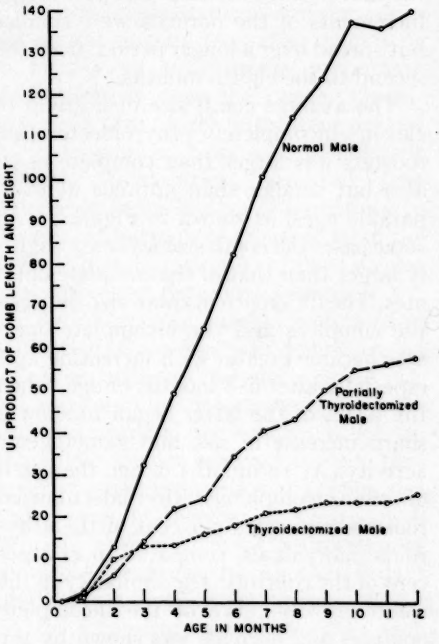

FIG. 23.—Comb size of normal and thyroidectomized males.

After 2 months of age the percentage ratio of the comb size of thyroidectomized males to the size of the normals exhibited a progressive decrease. The reduction in this ratio after 2 months of age is correlated with increased gonad activity in normals, which results in a rapid growth of the comb.

There was a distinct difference in

[4] Based on the product of comb length and height which has been found to have the closest correlation with comb weight (Jones and Lamoreux, 1943).

comb growth rates between thyroidectomized and normal roosters. Until 9 months of age, athyroidic roosters had attained a greater percentage of their 12-month size than had the controls (Fig. 23). The largest percentage of comb growth increments was exhibited by the thyroidectomized males during the first to third months. The percentage increments of the normals were smaller but spread over a longer period, from the second to the eighth months.

The average comb size of a group of eleven incompletely thyroidectomized roosters was larger than complete operates but smaller than normals at comparable ages, as shown in Figure 23. In some cases the comb size was only slightly larger than that of the complete operates. The difference in comb size between the complete and the incomplete operates became greater with increasing age, especially after 6–8 months of age, when the testes of the latter began to show a sharp increase in size and gametogenic activity. At 12 months of age the comb of the incompletely thyroidectomized roosters was 225.9 per cent of the athyroidic individuals, compared to 41.4 per cent of the controls. The similarity in the comb growth curve of the incomplete operates and normals was shown by the constancy of their ratio of comb size after 5–6 months of age and the similarity of their percentage of 12-month size increments.

2. HORMONE THERAPY

The androgens (Domm, 1927; Gallagher and Koch, 1929) and thyroid secretions (Crew, 1925; and others) have been shown to have a growth-stimulating effect on head furnishings in normal fowl, while estrogens were found to have none (Domm and Gustavson, 1929). Since thyroid secretions exercise a growth-stimulating effect on the comb, it was judged important to investigate whether the athyroidic condition affects the comb response to injections of thyroxin or steroid hormones.

a) Thyroid hormone.—Following thyroxin administration in thyroidectomized roosters, there was an increase in the size and vascularity of the comb. Within 4–6 days after the administration of 0.1 mg. thyroxin, the comb attained a 4.0–5.3 U.[5] increase in size. The increase persisted for 18–20 days after the injection. With the administration of a larger dose, as four doses of 0.1 mg. or of 0.15 mg. at 6-day intervals, the comb revealed growth within 2–4 days. There was a growth of 6.4–9.3 U. at 12 days after the first injection. A total increase in comb size of 14.0–14.6 U. was attained at 6–8 days after the last injection. The increased comb size was discernible until 26–30 days after cessation of medication. With an alternate-day injection of 0.05 mg. thyroxin for 30 days, which provided a more constant and longer period of medication, there was a 14.6 U. increase in comb size at 4–6 days after the last injection. An increase was revealed within 4 days after the first injection and persisted for over 30 days after the last injection. Biopsies for examination of gonads were omitted on these birds, so as not to interfere with the recording of other observations. The degree of comb response was proportional to the dose administered. Comb growth was also reported by Greenwood and Chu (1939) after feeding desiccated thyroid to thyroidectomized hens.

b) Estrogenic hormone.—Administration of alpha-estradiol benzoate in thyroidectomized roosters was generally followed by a decrease in the size and vascularity of head furnishings. With the daily administration of 5.0 γ of estradiol per 100 gm. body weight for 7 days (total

[5] Based on product of comb length and height.

daily dose, 0.059 mg.), there was a 3.6 U. decrease in comb size within 20 days after the start of injections. A reduction in comb size was evident within 4–6 days after the first injection with this and higher doses. The comb showed no increase in size until 20 days after the last injection. With a daily dose of 10.0 γ per 100 gm. body weight for 20 days (total daily dose, 0.109 mg.), a decrease of 4.6 U. occurred within 24 days. A maximum decrease of 7.6 U. was attained at 33 days after the first injection. The comb showed no increase in size for over 25 days after cessation of medication. At the termination of the daily administration of 16.5 γ per 100 gm. body weight for 22 days (total daily dose, 0.199 mg.), there was an 8.6 U. decrease in comb size. A maximum decrease of 14.9 U., attained at 36 days after start of treatment, persisted for over 30 days after its cessation.

No previous record was found of the effects of estrogens on comb growth or testis activity in thyroidectomized roosters. The decreased comb size observed in such individuals after estradiol administration corresponds to a similar effect observed in cockerels by Hoskins and Koch (1939). This may result from a pituitary gonadotropic inhibition, which would retard androgen secretion. Such an inhibition was reported following administration of estrogens by Moore and Price (1932) in mammals. The decrease in comb size observed in my experiments would therefore indicate that there is no cessation of secretion of the pituitary gonadotropic principle after thyroidectomy.

c) Androgenic hormone.—Within 7 days after beginning the daily administration of 1.0 mg. testosterone propionate for 5 days in a 2,660-gm. capon, there was a 15.4 U. increase in comb size. The same medication in a 1,200-gm. thyroidectomized rooster was followed by a 2.2 U. increase in comb size in 7 days. When this was followed by the daily administration of 2.0 mg. testosterone for 8 days, there was a further increase of 12.9 U., to make a total growth of 15.1 U. With the subsequent injection of 3.0 mg. for 5 days to this rooster, the comb revealed an additional growth of 4.1 U. for a total increase of 19.2 U.

The thyroidectomized rooster does not appear able to respond so quickly or so greatly to testosterone propionate medication as does the capon. Greenwood and Chu (1939), after treatment of thyroidectomized hens with a male sex hormone preparation, reported that "there had been no loss of sensitivity in the comb as a result of the removal of the thyroid glands." However, they do not present control data for comparison.

The influence of thyroid secretions in utilization of androgens is demonstrated by a comparison of the effects of individual and combined injections of thyroxin and testosterone propionate. When an athyroidic rooster received 0.5 mg. thyroxin at 3-day intervals and 1.0 mg. testosterone daily, there was a comb growth of 9.2 U. within 7 days. Continuation of the same treatment for another 7 days resulted in an additional increase of 10.3 U., for a total of 19.5 U. Another such individual receiving only the thyroxin medication revealed an increase of 12.9 U. An athyroidic male injected with only the testosterone showed a growth of 3.6 U. In a group of three capons, one was given 0.2 mg. every third day, the second 1 mg. of testosterone propionate daily, and the third both the thyroxin and the testosterone in the same dose. At 6 days after start of medication, the comb of the first had grown 0.4 U., the second 19.4 U., and the third 22.9 U. After 18 days of medication, the comb of the first showed no further increase, the second revealed a total growth of 52.7 U., and the third a

growth of 56.6 U. Thyroxin appears to increase the utilization of testosterone or has a synergistic effect with it on comb growth.

D. SPURS

It has been observed that spur growth in the capon exceeds that of the normal male, and, as a consequence, it is assumed that the testis secretion exercises a certain degree of inhibition on spur growth in the rooster (Domm, 1927). The hypogonadic condition of athyroidic roosters might therefore be expected to favor spur growth unless other factors, such as those associated with thyroid function, are involved. As far as the author is aware, no previous study has been made of spur development of chicks after thyroidectomy.

In the thyroidectomized rooster the spurs were "knoblike" and showed relatively little growth. They generally measured less than 1 mm. in length until the chicks were 7 or 8 months of age. The spurs of the normal rooster increased from a length of 1–2 mm. at 2 months to 9–10 mm. at 7 or 8 months of age. At 1–2 years the spurs of thyroidectomized roosters may grow to a range of 2–7 mm., compared to a range of 11–15 mm. in the normal. As also noted by Caridroit and Regnier (1944), the spurs became detached from the metatarsal bone and movable in the adult. The central part was composed of fibrous connective tissue instead of being calcified.

In the incompletely thyroidectomized rooster the spurs showed approximately the same size as in complete operates until 4–5 months of age. After this time the spurs of incompletes grew faster than in completely thyroidectomized roosters. The spurs of some incomplete operates measured 1 mm. in length at 7 months, while others measured 8–11 mm. and reached 16–19 mm. at 12 months. These variations were probably due to differences in the size of the remaining thyroid fragments.

From these results it would seem that the thyroid must be taken into account in any consideration of factors regulating or controlling spur growth and calcification.

E. BEHAVIOR

The characteristic pugnacity, crowing, and aggressive sexual behavior of the rooster is not revealed by the capon. The initiation and maintenance of aggressiveness and male mating behavior have been shown to be dependent on androgens (Domm, 1927; Allee, Collias, and Lutherman, 1939; Davis and Domm, 1941). Consequently, observations were made to determine whether the inhibition of gonad development in thyroidectomized roosters modified their mating behavior and if the athyroidic condition had any influence on their activities.

Though the capon is generally negative in its sexual behavior, it does not have the conspicuous lethargy characteristic of thyroidectomized roosters. Athyroidic individuals show a sharp decrease in activity when compared with controls. They generally have a droopy appearance and assume a squatty, rather than the characteristic upright, posture of normals. A similar lethargy was observed by Greenwood and Chu (1939) in their thyroidectomized fowl and by Nalbandov and Card (1943) after hypophysectomy. Completely thyroidectomized fowl stood or sat in their cages with their heads hanging or placed under their wings. Incomplete thyroidectomies generally assumed a more upright posture similar to normals (Figs. 1–3). Both groups manifested the usual excitability at feeding time.

Fighting and pecking in the cages was usually occasioned by the presence of

larger, incompletely operated birds, which would attack the smaller ones. In cages containing similar-sized thyroidectomized birds, relatively little fighting or pecking was observed. The incompletely thyroidectomized birds were more active than the complete operates, especially as regards fighting and pecking in their groups.

At 9–11 months of age some of the thyroidectomized roosters showed a slight strut, but rarely any courtship when tested.[6] No crowing was observed. Some incomplete thyroidectomies were observed not to have the typical courtship of normal roosters, such as chase, waltz, and circle, or treading of the female, which were revealed by other partial operates. Greenwood and Blyth (1929) and Schwarz (1930) reported treading by presumably incompletely thyroidectomized roosters. They report no observations on crowing. Simmons (1943) reported that a cockerel thyroidectomized at 5 days of age had not acquired the ability to crow when 82 days old.

Following injection of thyroxin or testosterone propionate, thyroidectomized roosters became more aggressive and belligerent. A similar effect was observed by Allee, Collias, and Lutherman (1939) after androgen medication of normal pullets and hens. After testosterone medication they would strut and waltz about a hen but showed no attempt to mount. No crowing was observed. Courtship, crowing, and treading can be induced by a similar dose of testosterone in normal capons (Domm, Davis, and Blivaiss, 1942). The administration of alpha-estradiol benzoate was observed not to have an effect on the behavior of athyroidic roosters, though larger doses were reported to have induced courtship and treading behavior in capons (Domm, Davis, and Blivaiss, 1942).

[6] This involved introducing a rooster that had been in isolation into a pen with a receptive hen.

F. FEATHER PATTERN (COLOR AND FORM)

1. PLUMAGE OF NORMAL MALE

Since the plumage of the Brown Leghorn is known from previous descriptions, a detailed account of its characteristics will not be presented; however, a brief survey of pertinent features will be helpful. The plumage of the male passes through four distinct stages, designated as "down," "chick," "juvenile," and "adult." These stages do not appear as sudden or abrupt changes but rather as gradual transformations, resulting from a slow continuous molt during the growth of the bird. While the down and chick plumages are quite similar in both sexes, the later stages are conspicuously different. The characteristic sexually dimorphic plumage does not become pronounced until the juvenile plumage appears.

A detailed description of the normal plumage of the Brown Leghorn may be obtained by consulting Domm (1927), Greenwood and Blyth (1929), or the American Standard of Perfection (1938).

2. PLUMAGE OF THYROIDECTOMIZED MALE

Thyroidectomy was observed to be followed by a replacement of the normal black, brown, red, and yellow melanins[7] by a light reddish-brown pigment (referred to as "tawny" by Ridgway, 1912) in all plumage stages, accompanied by a decrease in the number of barbules. These modifications resulted in a loss of the characteristic plumage pattern. Most of the feathers of thyroidectomized males

[7] Melanin refers to the amorphous black, brown, red, and yellow pigments believed to be formed by oxidation of tyrosine or 3,4-dioxyphenylalanine (Bodansky, 1938).

were entirely reddish-brown in color, noticeably elongated, narrow, tapered, and lacy,[8] and showed little or no web. The changes in feather form result from an elongation of the feather, a decrease in barbulation, an absence of hooks on barbules, and the abnormal position of some barbules, which lie nearly parallel to the barbs instead of extending laterally. The author found no previous reference to the latter condition in thyroidectomized roosters. These modifications interfered with web formation (Figs. 7–12).

The inhibition of molting in the thyroidectomized rooster was prominent and often resulted in the retention for periods much beyond the normal of a mixture of chick and juvenile feathers in juvenile birds and of juvenile and adult feathers in adult birds. At a time when normal birds have a complete adult plumage, thyroidectomized birds still conspicuously displayed juvenile feathers. The appearance of the adult plumage in thyroidectomized birds was usually hastened by plucking of the juvenile plumage. Operated birds kept in isolation frequently showed a nearly complete juvenile plumage at the time of normal adulthood. The lower feather growth rates of thyroidectomized roosters (see Table 2) also contributed to the delay in assumption of the adult plumage. The contrast between successive feather stages was not so marked in thyroidectomized individuals as in normals, owing to the greater similarity of feathers in the former.

The down and chick feathers which regenerated in areas plucked at the time of thyroidectomy showed evidence of hypothyroidism, both as to color and as to form. In all feather areas of the thyroidectomized cockerel there was either a complete loss or a partial replacement of the normal pigmentation by a reddish-brown pigment and a decrease in barbulation.

In the adult thyroidectomized rooster, operated on or before 10 days of age, body areas normally bearing black webbed feathers demonstrated the modifications in form and pigmentation most conspicuously (Figs. 7–10). Normally hackled or fringed feathers showed a further reduction or even complete loss of web (Figs. 8–9).

The normally black, solid-webbed breast feathers became entirely reddish-brown in color and narrow, elongated, and lacy. The web of the vane may be quite slender and confined to a thin strip along the rhachis or completely absent. The proportion of vane to fluff is greatly increased in feathers of thyroidectomized fowl, as compared to the normal. The fluff formed about one-third to one-quarter of the feather length rather than about one-half, as in controls. This was most conspicuous in the breast plumage. The body fluff, down, and shank feathers showed similar modifications.

The neck hackle exhibited a complete loss of the dark-brown or black pigment in the web. The orange-red of the fringe was blended into a reddish-brown, which extended to the rhachis. These feathers became nearly flufflike in form, owing to the extreme reduction or complete loss of barbulation (Fig. 8). Other hackled feathers with black and brown pigments, such as back, saddle, and wing-bow, showed the same type of modification. Some of the posterior saddle feathers became extremely elongated, approaching the length of the greater tail sickles of the normal rooster. Such feathers revealed virtually no barbulation and were entirely red-brown in color, similar to the other saddle feathers of athyroidic males.

The wing front feathers exhibited a

[8] The terms "lacy," "fringed," or "hackled" refer to the absence of barbules on barbs so as to form an area of free bristles.

TABLE 2

AVERAGE FEATHER-GROWTH MEASUREMENTS

(In Mm.)

Feather Age (Days)	2 Normal Roosters				2 Capons				2 Bilateral Poulards			
	Post. Br.	Ant. Br.	Back	Saddle	Post. Br.	Ant. Br.	Back	Saddle	Post. Br.	Ant. Br.	Back	Saddle
	Length											
16	13.2	11.5	9.2	9.6	11.6	9.4	7.1	8.6	13.3	11.9	11.0	7.6
24	33.1	30.7	24.6	22.3	33.7	30.1	22.3	21.4	34.4	31.2	30.0	22.1
36	60.9	56.6	52.6	43.4	64.8	57.4	51.3	44.2	64.1	57.6	57.0	50.0
48	83.0	74.4	75.7	63.9	92.8	79.6	76.7	67.6	86.5	74.4	75.0	78.5
60	94.9	79.4	85.4	85.4	.....	.....	.....	.....	87.3	78.2	80.5	103.8
80	100.8	.....	.....	118.6	.....	.....	.....	.....	.....	.....	.....	.....
	2-Day Growth Increments											
12–24	4.7	4.2	3.4	3.2	5.0	4.5	3.2	3.1	4.8	4.3	4.1	3.2
14–24	.....	.....	.....	.....	.....	.....	.....	.....	5.1	4.6	4.5	3.4
24–36	4.6	4.3	4.5	3.5	5.2	4.5	4.8	3.8	4.9	4.4	4.5	4.6
36–48	3.7	2.9	3.8	3.4	4.7	3.7	4.2	3.9	3.7	2.8	3.0	4.7
48–60	1.9	0.8	1.7	3.6	.....	.....	.....	.....	1.8	0.6	0.9	4.2

Feather Age (Days)	Thyroidectomized Roosters							
	4 Completes				3 Incompletes			
	Post. Br.	Ant. Br.	Back	Saddle	Post. Br.	Ant. Br.	Back	Saddle
	Length							
16	2.7	1.6	1.7	3.2	2.5	1.9	2.5	3.6
24	9.6	5.5	6.1	9.4	10.0	8.1	8.4	10.2
36	22.4	16.7	17.1	20.9	24.7	22.3	22.6	24.4
48	34.1	28.0	28.5	31.8	39.8	37.3	36.2	38.9
60	45.8	38.1	39.8	41.9	54.3	51.8	50.0	53.6
72	56.4	46.2	50.7	51.8	67.6	63.5	62.9	68.1
80	63.1	51.1	57.2	58.9	75.6	68.5	69.5	77.4
	2-Day Growth Increments							
14–24	1.62	0.97	1.0	1.44	1.7	1.4	1.4	1.3
24–36	2.13	1.86	1.85	1.92	2.4	2.3	2.3	2.3
36–48	1.95	1.88	1.88	1.82	2.5	2.5	2.2	2.4
48–60	1.93	1.88	1.88	1.68	2.4	2.4	2.3	2.4

loss of their black melanin and solid webbed vane, becoming reddish-brown in color and lacy in appearance. Wing coverts showed a loss of black melanin, which was replaced by a reddish-brown pigment and a tendency to become narrow and pointed. The vane was a uniform reddish-brown in color, narrow, and had a pointed apex. The web, though not fringed or lacy, was frayed and not well formed on its edges.

The primary flight feathers became narrower (Fig. 11). They displayed a replacement of the normal black melanin by an increased deposition of tan or reddish-brown pigment to form a band of reddish-brown pigment twofold or threefold wider than normal on the edge of the outer vane. A further slight deposition of reddish-brown pigment to form a band 2–3 mm. in width was observed on the edge of the inner vane. The secondaries were slightly narrower and more pointed than normal (Fig. 12). There was a conspicuous increase in the deposition of reddish-brown pigment, which replaced the normal black pigment. The entire outer vane assumed a reddish-brown color, instead of only a band of a quarter to a third of its width, as in the normal. The edge of the inner vane, which is normally entirely black, became reddish-brown in color. In the more medial secondaries, the entire inner vane also assumed a reddish-brown color. This type of modification in the flight feathers was also noted by Greenwood and Blyth (1929). The web of the secondaries, though not fringed or lacy, was not so well formed as in the normal, being frayed on its edges.

The normally broad-webbed tail coverts became markedly slender and tapered. The sickles displayed a narrower web and were considerably shortened in length. Both revealed a replacement of black melanin by a scattered deposition of reddish-brown pigment, especially at the margins and base of the vane. A decrease in barbulation was usually limited to the base of the vane.

The incompletely thyroidectomized roosters exhibited various gradations in modifications of plumage from the normal to those typical of complete thyroidectomy. They may be divided into two groups as to degree of modification. Group I included those which manifested, throughout, nearly all the pigment changes typical of complete thyroidectomy but not so extensive a degree of decreased barbulation. The feathers of Group I birds displayed characteristics similar to those described by Greenwood and Blyth (1929), Parkes and Selye (1937), and Fraps and Burrows (1939) following thyroidectomy, and by Hill and Parkes (1935*a*) and Nalbandov and Card (1943) following hypophysectomy. Group II included those which sporadically revealed the pigment and barbulation modifications typical of complete thyroidectomy in a section of some of the feathers, thus indicating a temporary athyroidic condition. The fluctuations in pigmentation and barbulation followed removal of thyroid rests by repeated operations.

In both groups of incompletely thyroidectomized cockerels, the more advanced development of the plumage compared to athyroidic cockerels was conspicuous. The modifications observed were generally similar to those in the adult. The changes in pigment were not so conspicuous here as in the adult, since the juvenile plumage normally has more reddish-brown pigment than the latter. The juvenile modifications will be described with those of the adult, in view of their similarity.

The breast feathers of Group I incompletely thyroidectomized roosters showed a nearly complete replacement of the

black melanin by reddish-brown pigment, but the decrease in barbulation usually was limited only to the edge of the vane. Their shape and form, therefore, were similar to the normal. The apex of these feathers frequently showed a small patch of black melanin, which was larger in the posterior than in the anterior breast feathers. The body down, fluff, and shank feathers exhibited similar pigment changes, but no further significant reduction in barbulation. The reddish-brown pigment which replaced the black melanin was darker in these cases than in completely thyroidectomized fowl. The basal fluff portion of the vane was slate or black in color, similar to the normal.

The neck hackle generally revealed a loss of the dark-brown or black pigment of the web, the entire feather becoming reddish-brown in color. There was no significant decrease in the width of the web or vane, which normally is quite narrow, or a change in the orientation of the barbs. The other hackled dorsal feathers, such as the back and saddle, showed a slight reduction in barbulation. The replacement of their black and red melanins by reddish-brown pigment was extensive but not complete.

The wing front, wing-bow, and wing coverts revealed a loss of black melanin which was replaced by reddish-brown pigment. However, there was no significant decrease in barbulation, the web remaining well formed. The primary flights were black with traces of tan on the edges of the outer vane, while the secondary flights showed only a slight increase in the width of the reddish-brown band on the edge of the outer vane. The remainder of the vane in the latter was black and had a well-formed web.

Tail coverts and sickles revealed no modification of their normal black pigment or barbulation. However, both had a narrower vane and were shorter than normal.

In Group II incompletely thyroidectomized roosters, the modifications generally appeared only in transverse segments of the feather rather than throughout, as in Group I. The breast revealed a loss of black melanin in varying amounts, which was more extensive in the anterior than in the posterior breast feathers. No significant modifications were observed in many of the feather areas. The maximum degree of modification was usually observed in the feathers regenerating after a biopsy, in which thyroid tissue was removed. In individual feathers a greater degree of modification was generally manifested on the edge of the vane than medially. This results from the fact that the apex of the barb has a higher threshold for hormonal modification than the base (Lillie and Juhn, 1932).

In cases in which the athyroidic condition had prevailed for a considerable period, the entire plumage was reddish-brown in color, narrow, elongated, and lacy, except the remiges and rectrices. The change in pigmentation usually extended over a larger area of the vane than did the loss of barbules. These modifications in plumage were, in general, proportional to the amount of thyroid tissue present at biopsy or post-mortem.

3. HORMONE THERAPY

a) Thyroid hormone.—Increased barbulation and black melanin formation have been reported following the feeding of desiccated thyroid (Greenwood and Blyth, 1929), as well as the injection of thyroxin (Emmens and Parkes, 1940) to thyroidectomized fowl. However, no data are available concerning the response of graded doses of thyroxin or the minimal dose necessary for the production of a normal feather. The effects of a single

dose of thyroxin and multiple doses at 6-day intervals have been studied.

The first visible effect of the administration of a single injection of 0.1 mg. thyroxin in a completely thyroidectomized rooster on the twenty-fourth or thirty-second day after plucking[9] was an increase in barbulation, followed by a black melanin deposition in the web of breast, back, and saddle feathers (Fig. 13). The increased barbulation and melanin deposition formed a band across the growing feather which varied in length and width in different areas. The effect of this and higher doses was readily discernible within 24–48 hours after injection by a study of split feather-germ preparations.[10] The melanin deposition continued for 4–6 days and formation of barbules for 8–10 days subsequent to the injection. A widening or broadening of the vane occurred in the feather area affected by the injection. The effect of such an injection was more conspicuous in the anterior than in the posterior breast feathers, as revealed by the length and width of the black webbed area. In the saddle a conspicuous, long, spindle-shaped area was formed by the increased barbulation and dark-red or black pigment. Proceeding posteriorly from its apex, the spindle shows a rapid widening, followed by a gradual basal taper. The longer duration of the effect of this dose in the saddle than in the breast was shown by the greater length of the webbed area.

The administration of a larger dose, such as two injections of 0.1 mg. thyroxin, each at 6-day intervals starting on the twenty-second or thirtieth day after plucking,[11] resulted in increased barbulation and melanin deposition for a longer period and in a larger modified area than in cases receiving but a single injection. In the feathers observed, there was no complete reversion to the typical athyroidic condition between the two injections. This was shown by the formation of a continuous band of black pigment and increased barbulation between the areas of maximum modification, which develop with each injection. This indicates at least a partial persistence of the effect of one such dose for at least 6 days. The area of barbulation and black pigment formed in the feathers after administration of the second dose was wider than the maximum of the first injection. This reaction, especially the increase in barbulation, was most conspicuous in the saddle. This dose and subsequent higher ones appeared to have a cumulative effect, since there was an increasing degree of modification with repeated doses, as revealed by the increased black melanin and barbulation of the saddle feathers.

With the administration of four injections of 0.1 mg. thyroxin each at 6-day intervals starting on the twenty-second day, the part of the feather developed after the second or third injection was typical of a normal rooster as concerns shape, barbulation, and color across the entire width of the vane (Fig. 14). While the tips of the breast feathers were reddish-brown, narrow, and lacy, their bases were solid black, broad, and webbed. The back feathers became broader and showed an excess of black, as compared to the normal rooster. The

[9] Since growing feathers are known to record hormone modifications, feathers were plucked before their administration. In this way, regenerating feathers capable of modification by the hormone were available.

[10] The sheath of a plucked feather papilla is split, which allows the unemerged feather to be spread flat on a slide for observation.

[11] The period of injection is given as the number of days which the feathers under observation have been regenerating. See Table 4 for details of the period of injection.

saddle exhibited a wider webbed area and a darker reddish-brown to black pigment than is normally present here.

While feathers displaying typically normal male characteristics on the entire vane (back and saddle) or a part of the vane (posterior and anterior breast) had been obtained, entirely normal feathers in all areas had not been produced with any of the previous doses. With the administration of fifteen injections of 0.05 mg. thyroxin each on alternate days from days 2–30, typically normal feathers were produced in the anterior and posterior breast areas (see Fig. 15). The breast feathers had broad round tips, were broad and fully webbed and solid black in color. The absence of reddish-brown pigment at the tips indicated that the entire feather developed under the influence of an effective dose of thyroxin. The back and saddle revealed barbulation and melanin deposition far in excess of normal. The tips of the back feathers had solid webs and showed more black melanin than normal. The web of the saddle extended the entire width of the feather and showed a broad area of black melanin.

A larger dose of thyroxin was required to produce normal color and form in the faster-growing feather areas, such as the posterior and anterior breast, than in the slower-growing areas, such as the back and saddle.

b) Estrogenic hormone.—The feminizing action of estrogens on the plumage of the Brown Leghorn is well known through the studies of Lillie (1942) and his associates and others. However, very little was known about the action of this hormone on the singularly modified plumage of thyroidectomized fowl. We were, therefore, interested in studying the possible role of the thyroid in the feminizing action of estrogens by observing their effects on the athyroidic plumage of thyroidectomized roosters.

In the normal rooster and capon, daily administration of 23–36 γ[12] of alpha-estradiol benzoate[13] for two successive days, starting on day 14, was followed by the development of female plumage characteristics in growing feathers. A diamond-shaped, salmon-colored area, 5–8 mm. in length, was formed across the width of the vane on the anterior breast feathers. The back and saddle feathers developed a wider webbed area with a brown mottled pigment (Fig. 16). These modifications were similar to those observed by Lillie and Juhn (1932) following the administration of 7–8 r.u. of estrogen per 100 gm. of body weight.

The dose of estrogen necessary to produce henny plumage is proportional to the feather growth rate, so that the more rapidly growing feathers require a greater concentration because of their higher threshold than do more slowly growing feathers. Consequently, one might expect a lower dose in general to be required for feminizing of plumage in thyroidectomized roosters because of their lower feather growth rates (see Table 2). To determine the minimal amount of estrogen necessary to modify plumage pattern in thyroidectomized roosters, two successive daily injections of between 10 and 41 γ of estradiol were made in three adults. No significant modifications in the color or form of the feathers were exhibited by any of these individuals, even though the doses administered in each in terms of body weight were relatively much higher than required for feminization of plumage in the rooster or capon.

The daily administration of 30 γ of es-

[12] See Table 5 for dose per 100 gm. of body weight, on which all dosages are based.

[13] Referred to hereafter as "estradiol."

tradiol for 15 days[14] was accompanied by only a slight modification in the breast but more extensive modifications in the back and saddle feathers (see Fig. 17). The anterior and posterior breast feathers exhibited a slight broadening of the web due to increased barbulation but no conspicuous increase in width of the vane, which remained hackled. The back feathers revealed an extension of barbulation but remained hackled. The vane became broader as a result of the assumption of a more obtuse angle between the barbs and rhachis and the lengthening of the barbs. The web showed a small amount of brown and black mottling. The saddle showed a slight broadening of the vane and a distinct increase in the width of the web. The web revealed a deposition of black melanin in varying amounts to form a distinct dark mottled pattern. These modifications became evident by the examination of split feather-germ preparations, 3–5 days after injections had begun. The greater response of the saddle to this and higher doses is probably the result of a lower threshold, as observed by Juhn, Faulkner, and Gustavson (1931) and Lillie and Juhn (1932) in the capon.

With the administration of higher daily doses of estradiol, such as 60 γ for 7 days, modifications of a similar character were observed. The saddle exhibited a marked increase in barbulation and black melanin, which showed some mottling. However, these feathers remained narrow and hackled. The back feathers showed similar modifications, though to a lesser degree. The breast also showed a slight increase in barbulation but no black melanin or mottling.

A marked modification of the breast feathers resulted from the daily administration of 109γ of estradiol for 20 days (see Fig. 18). Their apical ends revealed not only additional barbulation and broadening of the vane but also black melanin in a mottled pattern on a reddish-brown background. The amount of black melanin deposited was variable, though its presence was consistent. The back feathers showed extensive broadening, increased barbulation, and black and brown melanin in a mottled pattern. The saddle exhibited a widening of the web along the rhachis but remained narrow and retained a hackled margin. The web showed a more extensive deposit of black melanin, in a somewhat mottled pattern, than did the other feather areas. This pigmentation was much darker than that of the normal female.

With a larger daily dose of estradiol, such as 194 γ for 22 days, these modifications were not markedly enhanced in the breast feathers (see Fig. 19). In back feathers, and especially the saddle, hackling was generally lost, though the vane was not extensively broadened. This was the largest dose of estrogen administered, and it produced the greatest degree of barbulation and black and brown melanin formation.

For comparison with the complete operates, a Group I incompletely thyroidectomized rooster was treated daily for 8 days with 75 γ of estradiol, followed by 125 γ daily for 10 days. A greater degree of feather modification was produced than with the larger dose administered above in an athyroidic rooster (Fig. 20). The breast feathers revealed a larger amount of black melanin in a mottled pattern and a greater extension of barbulation than was found in any of the athyroidic roosters. The back feathers showed an extensive broadening of the vane, an increased barbulation, and a mottling of black and brown melanin. The saddle exhibited a widening of the web and vane but retained a narrow hackled region.

[14] See Table 5 for details of period of injection.

Its web showed a more extensive region of mottled black melanin than did the other feather areas. None of these feathers assumed the typical female pattern, though they had a more mottled pigmentation and a wider web than in the estradiol-treated athyroidic individuals.

Even with the large doses of estradiol used in completely and incompletely thyroidectomized roosters as compared with normal rooster and capon, a semblance of the typical female plumage was produced only in back and saddle feathers. These, however, did not exhibit the extensive broadening of the vane or the typical mottling of the pigment characteristic of the normal hen. With the doses used, the breast displayed an increase in barbulation, a broadening of the vane, and a deposition in the web of a dark-brown or black mottled pigment. The largest dose administered, even though much greater than that required to produce a typical female plumage in the normal rooster or capon, did not produce a plumage that was entirely henny in the athyroidic or nearly athyroidic rooster.

G. FEATHER GROWTH RATES

The feather growth rates of normal fowl have been shown to be modified by the administration of thyroid preparations (Torrey and Horning, 1925*a;* Domm, 1929*b;* Juhn and Barnes, 1931), as well as by estrogens (Domm, 1927; Domm and Gustavson, 1929; Juhn, Faulkner, and Gustavson, 1931) and by androgens (Juhn, Faulkner, and Gustavson, 1931). Therefore, we were interested in determining to what extent thyroidectomy influences feather growth rates in Brown Leghorn roosters and to see if the administration of thyroxin and estrogen in such individuals had the same effect on growth rates as follows their administration in normal individuals.

1. IN NORMAL FOWL

Since the characteristics of feather growth rates in Brown Leghorn roosters and capons are well known from previous investigations (Juhn, Faulkner, and Gustavson, 1931; Wang, 1945), a detailed analysis will not be presented. However, data are presented in Table 2 for comparison with that of athyroidic fowl.

The feather growth-rate increase at 10–12 days after plucking represents the transition in feather growth from Stage 1 (barb formation) to Stage 2 (beginning of shaft growth) (Lillie and Juhn, 1932). The average 2-day growth increment during the period of most rapid growth (days 24–36) was greatest in the posterior breast and least in the saddle. The difference in length relations of the feathers at the forty-eighth day compared with their length at maturity results from the sharp decrease in growth rate which occurs in all areas except the saddle after the forty-eighth day (see Table 2). Anterior breast and back feathers terminated their growth at 60–68 days and the posterior breast and saddle at 80 days after plucking. The feather lengths in the capon were greater than in the rooster, especially the posterior breast and saddle. Feather growth-rate averages for normal birds in the present study are based on feathers regenerated after each of four to six successive pluckings in a 1-year period.

No data were available on feather growth rates in bilateral poulards, shown by Domm (1927, 1929*a*) to develop the typical cocky plumage. In two bilateral poulards[15] (YW, 134) on which growth rates were observed, the feather lengths and growth rates were of the same order as those of the rooster and capon (Table 2). The absolute feather length and

[15] The bilateral poulards were obtained from a flock of such birds prepared and maintained by Dr. L. V. Domm.

growth rates were nearly identical with those of the capon.

2. IN THYROIDECTOMIZED ROOSTERS

Although a general retardation in feather regeneration has been noted in spontaneous (Landauer, 1929) and experimental (Parhon, 1924; Greenwood and Blyth, 1929) hypothyroid fowl, no observations were previously made on feather growth rates in the various body areas. Measurements of regenerating tail feathers in thyroidectomized Barred Plymouth Rock hen and rooster indicated a reduction in growth (Schwarz, 1930).

In the athyroidic rooster, the first stage (see p. 87) of feather regeneration was extended to 16–20 days after plucking, as compared to 9 or 10 days in the normal male. This estimate was based on the fact that an increase in growth rate occurred at this time in both types, which is characteristic for the transition point in feather growth between Stages 1 and 2. Regenerating feathers of the thyroidectomized male usually did not become measurable until 12–14 days after plucking, compared to 9 or 10 days in the normal. Feather regeneration was retarded to a lesser degree in the saddle, especially in the first stage, than in any of the other areas. This was readily demonstrable by the greater length of the saddle feathers, especially in the first 16 or 20 days of regeneration, when compared with the other feathers.

In four adult thyroidectomized roosters (Nos. 1355, 1362, 1392, and 3140) under observation, regenerating feathers continued growth with only a slight decrease as late as 80 days after plucking. This decrease appeared, first, in the anterior breast, which also terminates growth earliest in the normal male. At 80 days after plucking, the average lengths of regenerating feathers were similar to those of feathers in comparable areas of the normal rooster at approximately 34 days for anterior breast, 36–38 days for posterior breast and back, and 46 days for the saddle (see Table 2). The average 2-day growth increment during the period of most rapid growth (days 24–36) was reduced but greatest for posterior breast feathers, followed in decreasing order by the saddle, the anterior breast, and the back. These differences in growth rates were reflected in their respective lengths at 48 days after plucking, when the posterior breast was longest and the anterior breast was shortest, thus differing from the normals. Though the growth rate of the posterior breast later exceeded the more rapid growth of the saddle feathers in Stage 1 of regeneration, the other feather areas continued to be slower growing than the saddle. The normally slow-growing feathers, especially the saddle, were less affected by thyroidectomy, so that the saddle was of greater length than the back, which, in turn, was of greater length and faster growing for a longer period than the anterior breast. Though the posterior breast undergoes a much greater reduction in growth rate than the saddle, its growth rate still exceeded that of the saddle, especially when the feathers were over 48 days old.

A comparison of the mean feather lengths in two normal roosters, two capons, and two bilateral poulards with four thyroidectomized roosters at 48 days after plucking gives a P value in all cases of < 0.0004, which indicates a high statistically significant difference (see Table 3).

The average feather lengths and growth rates of three incompletely (Group I) thyroidectomized adult roosters (Nos. 940, 1231, and 3440) were greater than those of the complete operates. The order of growth-rate relations and ultimate feather lengths were similar to

those of athyroidic roosters (see Table 2). The accelerated feather growth rate of partially thyroidectomized roosters, though discernible in both the first and the second stages of feather growth, became more conspicuous with increasing age, especially in the second stage. The breast. The saddle had a demonstrably longer period of rapid growth than the other areas, as shown by its greater length at 80 days, as in the normal. The normally faster-growing feather areas had a greater percentage decrease in growth rate than the slower ones. The

TABLE 3*

FEATHER LENGTHS AT 48 DAYS OF AGE FOR THE FIRST PLUCKING

(In Mm.)

Bird	Type	Feather Areas			
		Posterior Breast	Anterior Breast	Back	Saddle
		L. S.E.	L. S.E.	L. S.E.	L. S.E.
1341	N	82.5±2.15	74.0±0.73	77.2±0.72	63.6±1.01
1820	N	82.4±1.40	74.5±0.68	74.9±0.89	62.6±0.45
1015	C	94.6±1.04	85.2±0.20	76.6±0.69	72.5±1.05
1021	C	87.8±1.34	79.5±0.62	77.8±0.67	75.0±1.46
YW	B	85.2±1.22	74.7±1.11	76.2±0.51	76.6±0.94
134	B	87.9±1.42	74.1±1.21	73.8±1.53	80.9±1.54
Mean		86.7	77.0	76.1	71.8
1362	T	33.9±0.94	27.4±0.51	30.0±0.68	32.3±0.61
1392	T	30.1±0.47	25.7±0.71	24.6±0.51	25.7±0.86
3140	T	35.7±0.87	26.6±0.41	26.1±0.71	33.4±1.17
1355	T	37.7±0.69	32.5±0.51	33.3±0.46	35.9±0.93
Mean		34.3	28.0	28.5	31.8
		t= 38.6 P=<0.0004	t= 38.4 P=<0.0004	t= 55.6 P=<0.0004	t= 20.2 P=<0.0004

* Following is the list of abbreviations:
N = Normal rooster
C = Capon
B = Bilateral poulard
T = Thyroidectomized rooster

L. = Mean length
S.E. = Standard error of mean

first stage of feather growth extended to 18–22 days of age. The saddle length exceeded that of the posterior breast until 28–32 days, and equaled or was slightly less until 44–48 days. At 48 days the average length of posterior breast was greatest, followed in a decreasing order by the saddle, anterior breast, and back. In the period of rapid growth, 24–36 days after plucking, the average 2-day increment was most rapid for posterior

thyroid section available did not appear adequate for a differential stimulation of feather growth rates to their tract-specific limits.

3. HORMONE THERAPY

a) Thyroid hormone.—The administration of desiccated thyroid (Torrey and Horning, 1925*a;* Domm, 1929*b;* Jaap, 1934) or thyroxin (Larionov, 1931; Lillie and Juhn, 1932; Hardesty, 1935) in nor-

mal fowl has been shown to accelerate feather growth. In view of the stimulating effect of thyroxin in normal fowl, experiments were performed to investigate whether a similar reaction would occur in athyroidic birds. Since there was a conspicuous decrease in feather growth rates following thyroidectomy, studies were made to determine the minimal doses necessary for acceleration to their normal levels. A series of single and multiple doses of thyroxin was administered to athyroidic individuals after the plucking of appropriate feather areas. With the concentrations used, black melanin deposition and barbulation were increased to varying degrees in all areas. No toxic effects, which are readily manifested by a blanching of feathers, were visible.

In the thyroidectomized rooster, the injection of 0.1 mg. of thyroxin was followed by a sharp increase in the growth rate of feathers for 10–12 days, returning to normal 4–8 days later (14–20 days after injection) (see Table 4). The stimulating effect of this and higher doses could be observed within 2 days after administration. The average 2-day growth increment for the period of 6 days following injection was most rapid in posterior breast feathers and least in back and saddle. While these growth rates were higher than those of the untreated thyroidectomized rooster, they were not so high as those of the normal rooster.

With the administration of two doses of 0.1 mg. thyroxin at a 6-day interval, the 2-day growth increment for the 6-day period following the second injection showed a further acceleration (see Table 4). A comparison of the growth rates attained when the same dose was administered at different feather ages revealed a higher rate when the injections were made on days 30 and 36 after plucking than earlier. This may be due to the greater responsiveness of feathers known to prevail at this age.

Greater stimulation was obtained by four injections of 0.1 mg. thyroxin at 6-day intervals (see Table 4). During the period of 6 days following the second injection, the feather growth rates represented the maximum obtained for the posterior breast and the near maximum for the other regions. The growth rate of the posterior breast feathers was closer to normal than the other areas. During the 6 days after the fourth injection, the back and saddle continued to show an increase in growth rate, while the breast showed a decrease. The anterior breast had a sharper and more rapid decrease in growth rate following cessation of medication than did the posterior breast.

The continued small increase in growth rate of the back and saddle after the breast had reached its maximum with this dose and higher ones resulted from their longer period of normal growth and lower threshold, which allows a longer period of response. The feather growth acceleration following thyroxin administration persisted for 10–14 days after the last injection. This period is somewhat dependent upon the age of the feathers at the last injection, a factor known to influence their responsiveness.

The maximum growth rates attained following the administration of four injections of 0.15 mg. thyroxin, each at 6-day intervals, were equal to or slightly less than that obtained with four successive injections of 0.1 mg. thyroxin (Table 4). The attainment of a lesser growth rate in the anterior and posterior breast feathers here than with 0.1 mg. thyroxin injections probably resulted from the fact that these injections were begun at a sufficiently later feather age to encounter the period of decreased responsiveness known to prevail toward the end of

the growth period of a feather. The back and saddle, however, reached a higher growth rate here than with previous doses. This may result not only from a greater stimulation with a higher dose but also from the continued responsiveness of slower-growing feathers at the later stages of growth. The average growth increment of the saddle feathers after the third injection equaled or exceeded the normal rate.

Since normal feather growth rates were not obtained throughout with the thyroxin doses employed, fifteen alternate-day injections of 0.05 mg. thyroxin were administered (Table 4). This procedure provided a more constant and longer period of medication. The administration of this dose was followed by stimulation of feather growth rates to the normal or even greater levels.

The time of appearance of the feather

TABLE 4*

EFFECT OF THYROXIN ADMINISTRATION ON FEATHER GROWTH RATES IN THYROIDECTOMIZED ROOSTERS

Bird	Dose (Mg.)	Inj. Days A	Feather Areas	2-Day Growth Rate Increments					
				Days Measured					
				Pre-Injection	Post-Injection				
1392.....	0.1	24 or 32		R −14–0	0–6	0–12	12–28		
			Post. br.	2.2	3.3	3.2	2.4	.....	.....
			Ant. br.	2.1	2.7	3.0	1.8	.....	.....
			Back	1.6	2.6	2.5	1.9	.....	.....
			Saddle	1.6	2.6	2.5	1.8	.....	.....
1392.....	0.1	22, 28 or 30, 36		R −10–0	0–6	6–12	6–14	6–18	
			Post. br.	1.7	3.0	4.2	4.1	3.7	.....
			Ant. br.	1.9	2.6	3.5	3.3	2.3	.....
			Back	1.8	2.6	3.6	3.4	2.3	.....
			Saddle	2.2	2.6	2.7	2.8	2.0	.....
1362[1].....	0.1	22, 28, 34, 40		R −10–0	0–6	6–12	12–18	18–24	28–38
				A 12–22	22–28	28–34	34–40	40–46	50–60
			Post. br.	1.3	3.1	4.4	4.4	4.1	2.2
			Ant. br.	1.1	3.1	4.0	4.2	3.8	1.5
			Back	0.9	2.8	3.9	4.1	4.2	2.1
			Saddle	1.5	2.7	3.0	3.0	3.3	2.2
1362[1].....	0.15	26, 32, 38, 44		R −10–0	0–6	6–12	12–18	18–24	
				A 16–26	26–32	32–38	38–44	44–50	
			Post. br.	1.5	3.3	4.3	4.0	3.9	.....
			Ant. br.	1.2	3.5	3.8	3.9	4.0	.....
			Back	1.1	2.9	3.7	3.7	4.1	.....
			Saddle	1.9	3.1	3.2	3.3	3.6	.....
1392[1].....	0.05	2–30, alt. days		R 10–14	14–24	14–34	36–46		
				A 12–16	16–26	16–36	38–48		
			Post. br.	.....	3.4	5.4	5.0	2.3	.....
			Ant. br.	.....	3.0	4.8	4.5	1.1	.....
			Back	.....	2.7	5.0	4.5	1.6	.....
			Saddle	.....	3.2	3.8	4.1	2.9	.....

* Following is list of abbreviations:
R = Days of measurement in relation to injection time
A = Actual days of injection based on feather age
[1] = Average of right and left feather areas

vane above the surface of the skin was similar to normal, though at comparable ages, such as 10–14 days, the respective feathers here were longer. The time of transition between the first and second stages of feather development (see p. 87) was still delayed when compared with the normal. It occurred 16–18 days after plucking, as indicated by the sharp rise in growth rate at this time. As with the lower doses, the normally faster-growing feathers were stimulated most and revealed the greatest growth rate. During the sixteenth to twenty-sixth days after injections had begun, the maximum 2-day feather growth increments were attained. The saddle continued to increase after this period, reaching its 2-day maximum growth rate, 4.1 mm., in the period between days 22 and 32. This thyroxin dose appeared capable of accelerating the initiation of the first and second stages of feather development and growth.

The feathers showed only a slight decrease in growth by the sixth day after cessation of medication with this concentration, after which a sharp decrease was noted. The period of sustained rapid growth after cessation of medication was less here than was observed in other cases. This was probably due to the greater length and maturity of the feathers and the consequent decreased reactivity. Saddle feathers exhibited a more gradual decrease in growth rate, which extended over a longer period than for the feathers of other areas. In this thyroxin-treated athyroidic rooster the accelerated regenerating plumage, except for the saddle, terminated its growth at an age closer to that of the normal rooster than in any of the other treated cases. This was probably related to the tract specificity for feather length.

b) Estrogenic hormone.—The feminizing action of estrogens on the pattern and growth rate of developing feathers is well known through the work of Domm and Gustavson (1929), Juhn, Faulkner, and Gustavson (1931), and others. Juhn, Faulkner, and Gustavson (1931) observed that, following the administration of appropriate doses of estrogenic hormone, anterior and posterior breast feathers of the capon showed a decrease in growth rate, while the back and saddle showed an increase, thus approximating the growth rates observed in the hen. In view of the observed feather growth-stimulating effects of estrogens, it seemed desirable to determine whether the thyroid played any role in this growth-promoting action. The slow-growing feathers of the thyroidectomized roosters were judged to furnish ideal material for the solution of this question.

Following two or more injections of 34 γ of alpha-estradiol benzoate[16] in the capon, feather growth rates became similar to those of the female in the respective feather areas as observed by Juhn, Faulkner, and Gustavson (1931) (see Table 5). The anterior and posterior breast feathers showed a decrease in growth rate, while the saddle revealed an increase. The rate of the back feathers was somewhat variable but generally lower.

Two successive daily injections of estradiol ranging from 10 to 39 γ of estradiol in three thyroidectomized roosters revealed no significant modifications in growth rate. The daily administration of 30 γ of estradiol for 15 days was followed in all areas by a 10–15 per cent increase in growth rates over untreated controls (see Table 5). The increase was greatest from the eighth to the sixteenth day after the start of medication. Conspicuous was the lesser increase in the growth rate of

[16] See Table 5 for dose per 100 gm. of body weight, on which the total dose has been calculated.

saddle feathers as compared to the other areas. It required a 4–6-day period of medication before a modification with this and higher doses was revealed. The growth rate returned to the untreated level within 4-6 days after cessation of medication.

The daily administration of higher doses resulted in a further acceleration of growth rates. With a dose of 49 γ for 20 days, the anterior and posterior breast showed an increase of 10–15 per cent, while the saddle revealed none (see Table 5 for the data of these experiments). The administration of 60 γ for 7 days resulted in a 25–30 per cent increase in feather growth rates. Further increases in growth rates to as much as 50 or 60

TABLE 5*

FEATHER GROWTH RATES FOLLOWING ADMINISTRATION OF ALPHA-ESTRADIOL BENZOATE

Bird	Type	Inj. Days (A)	Dose per Injection γ/100 Gm.	Dose per Injection Total γ	Feather Area	2-Day Growth-Rate Increments: Experimental						Control		
1211[1]	T	18–32 or 22–36	2.3	30	R	0–8	8–16	16–26	24–34			A 18–26	26–34	
					Post. br.	2.1	2.8	2.5	2.4	.....	.....	2.4	2.5	.....
					Ant. br.	1.5	2.9	2.8	2.5	.....	.....	1.4	2.3	.....
					Back	1.8	2.7	2.7	2.4	.....	.....	1.9	2.2	.....
					Saddle	1.1	2.3	2.2	2.0	.....	.....	1.8	2.2	.....
3140	T	26–40	3.5	49	R		0–8	4–14	8–16	16–26	26–34			
					A	20–26	26–34	30–40	34–42	42–52	52–60	26–34	34–44	
					Post. br.	2.7	2.9	3.0	2.7	2.4	2.2	2.4	2.5	.....
					Ant. br.	2.7	2.7	2.9	2.7	2.6	2.1	2.6	2.6	.....
					Saddle	2.8	2.5	2.6	2.3	2.1	2.0	2.6	2.7	.....
1362[1]	T	24–30	5.0	60	R		0–8	4–10	10–20					
					A	16–24	24–32	28–34	34–44			24–32	28–34	
					Post. br.	1.6	2.4	2.8	2.5	.....	.....	2.3	2.2	.....
					Ant. br.	1.2	2.3	2.7	2.5	.....	.....	2.0	1.5	.....
					Back	1.0	2.0	2.6	2.5	.....	.....	2.2	2.0	.....
					Saddle	1.9	2.2	2.3	2.2	.....	.....	2.0	1.9	.....
1362[1]	T	0–19 or 4–23	10.0	109	R	8–14	14–24	24–34	34–44			A 20–28		
					Post. br.	1.3	3.2	2.5	2.7	.....	.....	2.3	.....	.....
					Ant. br.	0.9	3.0	2.6	2.6	.....	.....	2.0	.....	.....
					Back	1.3	3.2	2.8	2.8	.....	.....	2.1	.....	.....
					Saddle	1.4	2.7	2.2	2.3	.....	.....	1.9	.....	.....
1392	T	0–21 or 4–25	16.5	194	R	14–24	24–34					A 14–24		
					Post. br.	1.9	2.8	.....	.....	.....	.....	1.2	.....	.....
					Ant. br.	1.8	2.6	.....	.....	.....	.....	1.3	.....	.....
					Back	1.9	3.0	.....	.....	.....	.....	0.8	.....	.....
					Saddle	1.9	2.7	.....	.....	.....	.....	1.3	.....	.....
1015	C	14,15	1.3	34	R		0–4	4–6						
					A	12–14	14–18	18–20				12–14	14–18	18–20
					Post. br.	4.3	3.9	6.3	.....	.....	.....	4.3	4.8	6.3
					Ant. br.	3.4	2.8	5.3	.....	.....	.....	3.4	3.8	5.3
					Back	2.3	2.0	4.4	.....	.....	.....	2.2	2.5	4.4
					Saddle	3.0	3.8	3.2	.....	.....	.....	3.1	3.2	3.9

*Following is list of abbreviations:
R = Days of measurement in relation to time of injection
A = Actual days of injection and measurement in terms of feather age
' = Right and left side feather areas measured
T = Thyroidectomized rooster
C = Capon.

per cent over controls were obtained with a dose of 109 γ of estradiol for 20 days. In the latter experiment the maximum 2-day growth rate was attained between the fourteenth and the twenty-fourth day after start of medication. In these experiments the greatest increase in feather growth rate was manifested by the breast and the least by the saddle.

The daily administration of 194 γ of estradiol for 22 days was also followed by an increase of 50–60 per cent in feather growth rates (see Table 5). The increased growth rate was discernible at 14–18 days of age, at which time the feathers first became measurable. A further sharp increase in growth rate was discernible after cessation of medication and persisted for 18–22 days thereafter. This may be a result of medication during the early development of the feathers (for 25 days, starting on day of plucking) when they are known to have a high degree of responsiveness. The saddle feathers were observed to maintain growth rates more similar to the posterior breast, which is different from the results with lower doses. The similarity of growth rate of the anterior breast feathers to that of the posterior breast and saddle was conspicuous. This would indicate a tendency for equalization of growth rates, as is typical of the hen or occurs after estrogen administration in the normal capon. While the feather growth rates showed an increase with the doses administered, the rates typical of the normal hen were not attained. The acceleration of feather growth may result from the characteristic stimulating effect of estrogens on feathers growing slower than the normal female rate.

IV. Discussion

The modifications observed following thyroidectomy in this investigation were, in general, more drastic than those reported by previous investigators. This is probably due to the more careful distinctions made between complete and incomplete thyroidectomy, as well as to the larger number of birds examined and the longer period of observation.

The retardation in body growth may result from a modification of pituitary function and basal metabolism, which is known to follow thyroidectomy and upon which growth is dependent. The pituitary cytology of thyroidectomized pigeons (Schooley, 1937) and chickens (Payne, 1944) is reported to be similar to that of dwarf chicks with undeveloped thyroids (Payne, 1944). An inhibition of the production and/or secretion of the pituitary growth-stimulating hormone has been reported following thyroidectomy in rats (Flower and Evans, 1924; Scow and Marx, 1945). Smith (1933) suggested that thyroxin stimulates growth in thyroidectomized rats by restoring normal pituitary function.

The metabolic level in birds has been shown to decrease following thyroidectomy (Marvin and Smith, 1943) and to be dependent upon the degree of thyroid activity (Riddle, 1932; Miller, 1938). In normal chicks, body growth can be stimulated by the oral administration of small doses of iodocasein (Irwin, Reineke, and Turner, 1943) or thyroxin (Simmons, 1943). Hammett (1926) suggested a growth-promoting action for the thyroid secretion by reason of its production of an increase in cell size in contrast to an increase in cell number by the growth hormone of the pituitary.

The thyroid appears to have a general calorigenic action, as well as a specific action on other endocrines. The simultaneous decrease in the thyroid hormone and the pituitary growth hormone leads to such a striking modification of body growth because of their synergistic action (Evans, Simpson, and Pencharz,

1939). Salmon (1936) postulated that the thyroid was responsible for development of the capacity to respond to other hormones. The larger size of incompletely thyroidectomized birds probably results from their higher level of pituitary growth hormone and its greater utilization as a result of the availability of some thyroid secretion.

Previous observation on thyroid-pituitary-gonad relations indicates both a direct and a secondary control of gametogenic activities by the thyroid. The administration of an adequate dose of desiccated thyroid (Crew, 1925; B. M. Zawadowsky, 1925*a*) or thyroxin (Aron and Benoit, 1934) to normal sexually quiescent birds or thyroidectomized birds (Winchester, 1939; Greenwood and Chu, 1939) leads to an augmentation of gonad activity.

The importance of the secretions of the anterior lobe of the pituitary in the development and maintenance of the activity of the gonads has been demonstrated by Domm (1937). A reduction in gonadotropic potency of the pituitary after thyroidectomy has been reported in mammals (Evans and Simpson, 1930; Van Dyke and Ch'en, 1933; and others). Smelser (1939) suggested that the decreased gonadal activity of thyroidectomized rats resulted from a decrease in the amount of gonadotropic hormone secreted into the blood. Benoit (1936, 1937) observed an increase in the gonad activity of thyroidectomized drakes after exposure to light. He considered this to result from the increase in pituitary gonadotropic activity known to follow such treatment (Benoit, 1935).

The decreased size of the seminiferous tubules and inhibition of gametogenesis following thyroidectomy would, therefore, seem to result from (1) an absence of thyroid secretion, which probably acts to stimulate tissue growth by its control of metabolism; (2) a decrease in secretion or potency of pituitary gonadotropic and growth hormones, known to follow thyroidectomy; and (3) a decrease in ability to utilize the available pituitary hormones as an aftermath of the reduced basal metabolism. Incompletely thyroidectomized roosters show a greater amount of testis growth and gametogenic activity than complete operates, owing probably to the fact that the former have thyroid secretion available and, consequently, an increased production and utilization of pituitary hormones. The degree of development is related to the amount of thyroid present. This has also been noted by others (Greenwood and Chu, 1939). These investigators also suggest an intermediary role for the pituitary in the inhibition of gametogenesis observed after thyroidectomy.

Since comb growth is known to be dependent on androgens (McGee, Juhn, and Domm, 1928; and others), it is logical to assume that the decreased comb size of thyroidectomized roosters may be the result of an insufficient androgen secretion or utilization in such individuals. However, the absence of a capon-like condition in these characters in such individuals indicates that androgen production or its utilization is never completely suppressed.

In the presence of thyroid secretions there is probably a stimulation of pituitary gonadotropic hormone and androgen secretion (Smith, 1933), as well as an increase in ability to use these hormones (Salmon, 1936). This accounts for the increase in comb size observed in this investigation after injections of thyroxin and after feeding of desiccated thyroid to thyroidectomized fowl by Greenwood and Chu (1939) and to normals by Crew (1925). The interrelation of thyroid and testicular secretions in comb growth is also shown by its greater size in partially

thyroidectomized roosters of this and previous investigations (Schwarz, 1930; and others). The stimulating influence of thyroid secretions was shown by the greater comb growth of capons (Simmons, 1943) and in capons as well as thyroidectomized roosters in my experiments after the simultaneous administration of thyroxin and testosterone than following either alone.

The reduced spur development following thyroidectomy cannot be considered to result from an absence of gonadal hormones, since estrogens inhibit initiation of spur growth, while androgens exercise a retarding effect on their development (Domm, 1927). The decreased spur growth in thyroidectomized roosters was probably a result of the combined effects of a reduced tissue metabolism and a deficiency of pituitary growth hormone which led to a reduction of general body growth (see above).

The slowing of nervous reactions and dulness of the sensorium occurring concurrently with the decrease in basal metabolism characteristic of hypothyroidic individuals (Thompson, 1942) probably led to the lethargy of thyroidectomized roosters. The lethargy of cretinism is treated by increasing the basal metabolism with thyroxin medication (*ibid.*). The absence of mating behavior and crowing in the athyroidic roosters may result in part from the general apathy of hypothyroidic individuals and possibly from an inadequate utilization of androgen.

Except for one short-lived case of a possible complete thyroidectomy reported by Greenwood and Blyth (1929), all previous observations on feather modifications in Brown Leghorn roosters and capons were probably based on incomplete operations. This is indicated by the lesser degree of pigment and barbulation modifications than that observed in the athyroidic males of my experiments and and by the ultimate reversion to normal plumage after the molt in many of the cases (Greenwood and Blyth, 1929; Parkes and Selye, 1937; Emmens and Parkes, 1940). The absence of black or slate pigment in the fluff portion of the feather was not previously noted. Most of the plumage modifications in preceding experiments were similar to that of Group I or Group II incompletely thyroidectomized roosters discussed in this paper (see pp. 82–83). Extensive debarbulation has been observed in some cases of toxic hyperthyroidism which was believed to result from an abnormal metabolism (B. M. Zawadowsky, 1925*a*, *b;* Cole and Hutt, 1928).

The differences in hormone threshold among the various feather areas or within individual feathers (Lillie and Juhn, 1932) are reflected in the degree of feather modification observed after thyroidectomy. The uniform and complete loss of normal pigments and barbulation in a particular feather or feather area indicates that the level of thyroid secretion was below that necessary for their production. The persistence of a slate-colored pigment in the fluff portion of some feathers or black melanin in parts of the primary and secondary flights and tail feathers in completely thyroidectomized roosters may indicate that the thyroid secretion is not necessary for its production in the feathers of these areas. Since the main tail feathers have a higher threshold for estrogens than other body areas (Domm, 1927) and inasmuch as the thresholds for thyroid generally conform to those for estrogens, one would expect a loss of black pigment in these feathers as in those of lower thresholds. At present no suitable explanation is available for the persistence of black pigment in the tail and flight feathers. Greenwood and Blyth (1929) and Emmens and

Parkes (1930) agree that the regionalization of feathers and differences in pigmentation and form within the individual feather are the result of their threshold differences.

The observed difference in hormone threshold in the bilateral halves of an individual feather has made possible the experimental production of a modification in only one half-vane of a feather (Lillie and Juhn, 1932). The presence of a dark, reddish-brown pigment on the outer vane of the normal secondary flight feathers would indicate that a higher level of thyroid secretion was necessary here for the production of black melanin than on the inner vane. The unilateral difference in the degree of modification obtained in these feathers after thyroidectomy (a greater loss of black melanin on the outer half-vane than on the inner one, which was replaced by reddish-brown pigment) seems to conform to this difference in threshold.

In partial thyroidectomies the hypothyroid modifications were frequently manifested as a "tipping" or "barring" of feathers in a manner similar to that noted by Domm (1927) in ovariotomized fowl. In all feather areas of incomplete operates there was a more extensive loss of black melanin than of barbulation, which indicates that a higher threshold of thyroid secretion was necessary for black melanin production than for maintenance of barbulation. This conclusion conforms with the data obtained from thyroid feeding (Domm, 1929*b*) or thyroxin injection (Juhn and Barnes, 1931; Lillie and Juhn, 1932).

The diversity in degree of alteration of the feather areas in partially thyroidectomized roosters resulted from an oscillation in the amount of thyroid secretion available owing to thyroid regeneration or removal and the differential in feather thresholds. Since faster-growing feathers have a higher threshold than the slower ones (Juhn, Faulkner, and Gustavson, 1931; Lillie and Juhn, 1932), more extensive and conspicuous plumage modifications occurred in the faster-growing posterior and anterior breast than in the slower-growing back and saddle feathers.

A variation in degree of modification was also observed within individual feathers of incomplete thyroidectomies by others (Greenwood and Blyth, 1929; Emmens and Parkes, 1940). These result from differences in the reaction thresholds within the individual feather (Lillie and Juhn, 1932) and variations in the level of thyroid secretion. Even when the level of thyroid secretion is kept constant for the period of a feather's axial growth, an unequal degree of modification occurred, probably as a result of threshold differences over the length of the feather. The development of a black melanic area at the apex of some feathers, otherwise hypothyroid, may be associated with the slow growth rate in the first stage of feather development (see p. 87), when these barbs are formed, while the development of black melanin at the base of these feathers is, no doubt, associated with the slower growth rate and the concurrently decreased threshold occurring at the approach of feather maturity. The decrease in threshold from apex to base of the barbs (Lillie and Juhn, 1932) results in the appearance of signs of thyroid deficiency at the apex of the barbs (margin of vane) sooner and to a greater degree than at their bases (rhachis area).

The area of increased barbulation following thyroxin administration usually exceeded black-pigment formation, which was in accordance with the higher threshold required for the production of the latter. The melanin and barbulation formed in the saddle were in excess of those normally present here, which indi-

cates that 0.1 mg. of thyroxin was above the minimal dose necessary to bring about development of the normal condition in this area. The spindle-shaped black melanic area formed about the rhachis after thyroxin administration illustrates the relation of thyroid level and feather threshold to the extent of modification. Proceeding posteriorly from its apex, the spindle shows a rapid widening, which indicates a rise in thyroxin concentration. The widest part of such a spindle is above its center, indicating a more rapid absorption than excretion. The basal taper of the spindle indicates a gradual lowering of the thyroid level. This reaction is in accord with the known rise in threshold of reaction from the base to the apex of the barbs (Lillie and Juhn, 1932).

The development of a cocky plumage in the capon and bilateral poulard indicates its independence of gonadal secretions (Domm, 1927, 1929*a*). The dependence of cocky plumage on a certain level of thyroid secretion for its maintenance has been suggested by Greenwood and Blyth (1929). This is supported by the reappearance of the normal plumage pattern in my thyroidectomized roosters following thyroxin administration.

The development of "female-like" characters, such as a sparse mottling and an extension of barbulation in the athyroidic feathers of the rooster following estradiol administration, rather than the dense mottling and solid-webbed vane such as develops in the normal rooster following such medication, reopens the question of the role of thyroid and ovarian secretions in development of the female plumage pattern in Brown Leghorn fowl. Do thyroid and ovarian secretions have a parallel, complementary, or synergistic effect in the development of female plumage?

Greenwood and Blyth (1929) believed that "the former (ovary) stimulates the latter (thyroid) to a higher level of activity than that present in the male, and so indirectly causes a hyperthyroid effect on feathers," as seen by the extensive barbulation in the hen plumage. "At the same time, it (ovarian secretion) modifies this condition by acting directly on the feathers and restricting deposition of melanin." The author is inclined to agree with Fraps and Burrows (1939) that this does not appear to be a valid conclusion, since the basal metabolism of the rooster (Mitchell and Card, 1927), as well as the male pigeon (Riddle, 1932), is generally higher than that of the female.

Fraps and Burrows (1939) suggested a complementary action between ovarian and thyroid secretions in the production of female plumage pattern. This was based on their obtaining an extension of black melanin followed by a blanching of the feather in a thyroidectomized capon after injection of large doses of theelin. The blanching effect is regarded as a typical toxic reaction in normal fowl. The modifications in the saddle feathers were less marked than in the posterior breast, which would not be expected on the basis of their thresholds.

In thyroidectomized roosters, thyroxin brings about the development of an area of solid black melanin and barbulation, whereas estradiol not only stimulates an extension of barbulation but also a mottling of black or brown melanin. When both thyroid secretions and estrogens are available, as in the hen, a female pattern is produced. The inability to produce this pattern in an estradiol-treated athyroidic rooster may indicate that the function of the thyroid is to increase the ability of the feather germ to utilize the estradiol. This conclusion is supported by a report of the development of the female pattern in an incompletely thyroidectomized capon following theelin administration

(Fraps and Burrows, 1939). Similarly, in my experiments a greater amount of barbulation and mottling of black and brown pigment was produced in an incompletely thyroidectomized rooster (No. 3440, p. 86), which had received only a small dose of estradiol, compared to that of complete operates.

My results on injection of alpha-estradiol benzoate in athyroidic roosters indicate a synergistic action between estradiol and thyroxin in development of the female pattern. The greatest degree of modification was observed in the slow-growing feather areas, which conforms to their lower threshold. The failure to develop a typical female pattern after estradiol administration may be due to (1) an inability of the feather germ to utilize adequately the hormone in the absence of thyroid secretions or (2) the fact that the estradiol may be excreted too rapidly to permit its utilization. In normal fowl the administered estrogens are utilized at a sufficiently rapid rate to be effective before they are excreted.

To account for the replacement of the normal pigments by a reddish-brown pigment after thyroidectomy, it is necessary to consider whether these pigments are the product of the same or of different melanophores. Though the plumage melanoblasts originate in the neural crest (Danforth, 1937; Dorris, 1939), they may undergo various modifications after migration to the dermis (Waterson, 1942; Foulks, 1943). Wang (1943) favors the "monophyletic theory" of pigment formation, in which only one type of melanophore is involved in the production of both black and red pigment, possibly under the differential control of the epidermal substratum. This would imply that the melanophores are dependent in some specific manner upon the feather-forming epidermis. Willier (1942) favors the view that both the red and the black types of melanophores are present. Whether black or red pigment is to be formed lies under control of the follicular epidermis. Hamilton (1940), from *in vitro* studies, found no evidence for the conversion of one kind of melanoblast into another. In order to yield red melanoblasts, a bird would need to have a genetic constitution for the synthesis of red pigment. Hamilton (1941) found two distinct cell types, but no intermediate types.

Ladebeck (1921) reported the existence of transitional forms from black to red in the granules of Brown Leghorn feathers. This would suggest that red-brown and black were successive stages in the process of pigment formation by a single type of melanoblast. Bohren, Conrad, and Warren (1943), however, could not conclusively find such a differentiation of granules either by morphological or by chemical criteria.

In normal (Lillie and Juhn, 1932) and thyroidectomized Brown Leghorns, melanophores producing one kind of pigment may be completely replaced by those producing another within less than 24 hours after the injection of hormone. Both before and after such a change the barb ridges may be densely packed with pigment cells. The promptness and completeness of the color changes elicited by hormones (as pointed out by Danforth, 1937) make it seem improbable that the hormones are merely acting selectively upon two types of melanophores previously determined with respect to type. Willier and Rawles (1944) have considered that the melanophore precursors are fixed as either black or red after entering the epidermis of the developing feather. Their reference to "precursor melanophore" may indicate that they believe it to occur at some time before the beginning of pigment formation. Wang (1943) has shown that the feather

papilla epidermis is the controlling factor for melanophore determination and that this is probably determined concurrently with other morphological criteria of tract specificity.

Since pigment alterations appeared concurrently with modifications in growth rate, a difference in metabolic rate may be involved. The influence of thyroid secretion on basal metabolism has been shown in the pigeon (Riddle, 1932; Marvin and Smith, 1943) and the sparrow (Miller, 1938). Lillie and Wang (1941) have suggested that the development of certain composite colors from a combination of breast and saddle half-papillae, which results from a replacement of saddle cells by those of the breast, might be associated with some intrinsic property related to the breast's higher growth rate. Faulkner (1932) proposed the theory that black pigment was formed in feathers whose growth rate exceeded a certain level, and that, below this rate, red pigment was formed. However, no explanation was offered for feathers whose pigment does not correspond to her growth-rate scale or those which have both black and red pigments.

A correlation between basal metabolism and plumage pattern is suggested by the close association of feather structure and pigment with growth rate. The rapidly growing feathers of the male breast have a black pigment on a wide and well-developed web. The slower-growing feathers, such as back and saddle, exhibit black and red pigment on a hackled vane. The saddle, which has the slowest growth rate, shows the greatest amount of red pigment and hackling. The administration of thyroxin in the rooster or capon (Lillie and Juhn, 1932) was followed by an increase of black pigment and barbulation in the back and saddle, concomitant with an increased growth rate. The administration of estrogen in the rooster stimulated an increase in feather growth rate, formation of black and brown pigment, and barbulation in back and saddle feathers (*ibid.*).

In the red-pigmented feather areas of normal and thyroidectomized fowl, a certain physiological substance or process necessary for oxidation of tyrosine or dopa into black melanin may be lacking in the follicles. Dorris (1939) suggested the existence of an "inhibitor" to the synthesis of black melanin. The production of such a substance by the male saddle epidermis may account for the absence of black here (Wang, 1943). Another possibility is that the chain reaction affecting the oxidation of dopa into black melanin ceases at an intermediate stage, at which the oxidation product is a red-brown pigment. This would be confirmed by establishment of the existence of intermediate granule stages between black and red, as reported by Ladebeck (1921). The latter may be the result of a mere quantitative difference, as, for instance, in the oxygen content of the oxidases (Wang, 1943).

The existence of a single stem cell which has the capacity to differentiate into either a black or a red melanophore is supported from a chemical point of view. A red-colored substance has been shown to be an intermediate product in the oxidative action of tyrosinase on tyrosine to black melanin. (Raper, 1932). However, Nickerson (1946) has obtained an *in vitro* oxidation of black melanin or dopamelanin to a product whose solubility and absorption spectrum are similar in some respects to those of natural red melanin. Figge (1940) has found the production of melanin from tyrosine by tyrosinase to be regulated by the oxidation-reduction potential of the substance added to the reaction mixture. A red pigment is formed at a lower redox potential than is black. He (*ibid.*) states

that "the normal pigmentary . . . . alterations associated with physiological changes or sex hormones . . . . are but visual evidence of a change in the oxidation-reduction potential of melanogenic centers in the cell."

The marked reduction of black pigment in thyroidectomized fowl may result from a decreased oxidation-reduction potential, a direct hindrance of the dopa or tyrosine oxidation in the absence of thyroid secretion, or the decreased metabolism and growth rates of the feather follicle. Faulkner's (1932) hypothesis would indicate that the absence of black pigment in athyroidic fowl is a result of the decreased feather growth rates. The simultaneous inhibition of black melanin production and feather growth rates after administration of thiouracil to normal fowl (Domm and Blivaiss, 1944, 1946) conforms to the proposed relation of plumage pigment production to basal metabolism and feather growth rates.

No conclusive data are available as to the existence of one or two types of melanophores. It appears feasible to assume that, in the absence of thyroid secretions, either (1) the pro-pigment in the melanophores can be oxidized only to the red-brown stage or (2) only those melanophores which have red-brown as their end-product are able to undergo oxidation. These reactions probably occur under the control of the epidermis.

The reduced feather growth rates of athyroidic individuals need to be considered on the basis of their reduced metabolic level and pituitary growth hormone secretion. Gonadectomy does not result in such a retardation as seen from the feather growth rates of capons and poulards. However, feather growth acceleration has been shown to follow thyroid or thyroxin administration in normal fowl (Torrey and Horning, 1925*a*; Domm, 1929*b*; Lillie and Juhn, 1932). Lillie and Wang (1940) have suggested a correlation between basal metabolic rate and the growth rate of feathers. The close relation between thyroid activity and feather growth was indicated by the development of feathers in chicks in correlation with an increase of thyroid activity as determined by secretory action and iodine content (Chaudhuri, 1928; Benozzi, 1932).

The more rapid growth rate of the posterior breast than of other feather areas in thyroidectomized roosters suggests the existence of a basic order of feather growth rates.[17] The fact that a similar relation occurs in normals suggests that their higher metabolism results in an acceleration of feather growth rates which is roughly proportional to the basic order observed in athyroidic males. The tendency toward an equalization of feather growth rates in thyroidectomized roosters is conspicuous. The existing differences may represent a response to a nonthyroid metabolic stimulant or the prevailing metabolic level. The more rapid growth rate of the saddle compared to anterior breast and back feathers may represent the response of a low threshold feather area to a small but constant level of stimulation.

The need for a larger dose of thyroxin to stimulate and maintain an increase in feather growth rates in the posterior breast than in the saddle emphasizes threshold differences. The apparent existence of a tract specificity for the rapidity of growth to which the feathers could be stimulated was shown by the saddle feathers. For instance, with doses of thyroxin in excess of the amount necessary for acceleration to its normal growth rate, the saddle would not exceed its normal rate or approximate the higher rate

[17] A similar order of feather growth-rate relations has been found in thyroidectomized hens (Blivaiss and Domm, 1944).

of the posterior breast. This may be due to an inability of the epidermal follicular cells of the saddle to exceed a certain metabolic level which would influence the growth rate.

Further clarification of the relations between plumage pattern and growth rates and gonad and thyroid secretions may be obtained from a study of effects of the simultaneous administration of thyroxin and estrogens or androgens in thyroidectomized capons and bilateral poulards. It is conceivable that the relation of basal metabolism to plumage pattern and growth rate can be observed by a study of the effects of a nonthyroid metabolic stimulant. A careful study of the alterations in pituitary and adrenal gland histology and in secretions after thyroidectomy might facilitate interpretation of some of the problems discussed in this paper.

V. Summary and Conclusions

1. A study was made of the relations between body growth, gonad function, and plumage pattern and growth rates in 98 complete and 154 incomplete thyroidectomized Brown Leghorn roosters.

2. Development of the gonads and secondary sexual characters of adult, completely thyroidectomized roosters was markedly reduced. The greater degree of development of partial thyroidectomized males compared to complete operates became more conspicuous with increasing age.

In athyroidic fowl no spermatogenic stages beyond secondary spermatocyte were observed as late as 22 months of age. The vas deferens remained relatively thin and straight.

With androgen injections, a lesser comb growth was revealed in athyroidic fowl than in capons. The comb of capons and athyroidic males displayed a more marked growth following the simultaneous administration of thyroxin and testosterone propionate than with either alone.

3. Following thyroidectomy, all plumage stages exhibited a replacement of normal pigmentation by reddish-brown pigment and a decrease in barbulation.

The male plumage pattern was produced in athyroidic roosters with an adequate dose of thyroxin without regard to testicular secretion.

The normal female plumage pattern was not produced by the administration of alpha-estradiol benzoate in thyroidectomized roosters, as is the case in normals. The female pattern appears to result from the synergistic action of thyroid and ovarian secretions.

The modifications of feather pattern conformed to the differences in thresholds of feather areas, as well as within individual feathers.

4. After thyroidectomy, the feather growth rates were reduced, but the growth of the posterior breast was most rapid for a longer period than the other areas, as in the normal.

The normal feather growth rates can be restored in thyroidectomized roosters by administration of thyroxin. A tract specificity appeared to exist as to the magnitude of stimulation obtainable by thyroxin administration, which corresponded closely to the normal feather growth rates.

The increase in feather growth rates following estradiol administration was accompanied by a tendency for the equalization of their absolute rates, as prevails in the normal hen.

5. Suggestions concerning the influence of the thyroid-pituitary-gonad interrelations on body growth, gonad function, and secondary sexual characters are discussed.

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PLATE I

FIGS. 1–3.—Adult normal and thyroidectomized roosters.

FIG. 1.—Adult normal rooster, six months of age, shows well-developed comb and wattles which are red and turgid, and prominent ear lobes.

FIG. 2.—Adult complete thyroidectomized rooster (No. 5614) at 10 months of age. Thyroidectomy completed at 10 days of age. Note the smaller body, squatty stance, and the rugged appearance of the plumage compared to the normal. The comb and wattles are much reduced in size, pale and less turgid than in the normal. The ear lobes are also smaller.

FIG. 3.—Adult incomplete thyroidectomized rooster (No. 3440) at 10 months of age. Original thyroidectomy completed at 9 days of age. Note the reduced body size compared to the normal, though larger than the complete operate. This bird displays a squatty stance and rugged plumage. The plumage is generally typical of thyroidectomy but also displays areas of normal pigment and form, especially at the tips and bases of individual feathers.

FIGS. 4–6.—Testes of adult fowl (magnifications: *a*, ×115; *b*, ×650).

FIG. 4.—Testes of adult normal Brown Leghorn (No. 1331), 6 months of age, showing tubules in full spermatogenesis.

FIG. 5.—Testes of adult thyroidectomized Brown Leghorn (No. 1471), 6 months of age, showing primordial germ cells and primary and secondary spermatocytes. Note compact arrangement of tubular and intra-tubular cells, typical of an immature testis.

FIG. 6.—Testis of adult thyroidectomized Brown Leghorn (No. 1362), 22 months of age, showing primary and secondary spermatocytes but no mature spermatozoa. Note compact arrangement of tubules and intra-tubular tissue.

PLATE II

FIGS. 7–12.—Types of homologous adult normal and thyroidectomized rooster feathers; in each pair, normal to left and thyroidectomized to right. Fig. 7, posterior breast; Fig. 8, neck hackle; Fig. 9, saddle; Fig. 10, wing coverts; Fig. 11, primary flights; Fig. 12, secondary flights.

FIGS. 13–15.—Photographs of feathers of adult thyroidectomized roosters after administration of thyroxin. (In all figures, *a*, posterior breast; *b*, anterior breast; *c*, back; and *d*, saddle.) Note the athyroidic condition of the feather tips in Figs. 13 and 14.

FIG. 13.—Injection of 0.1 mg. thyroxin on day 24 or 32 after feather plucking. Observe deposition of black melanin for a longer period in saddle than in other areas and the greater area of barbulation than pigment deposition.

PLATE III

FIG. 14.—Four injections of 0.1 mg. thyroxin at 6-day intervals, starting on day 22. The part of the feather developed after the second or third injection is typical of a normal rooster as to color and form. Observe persistence of a single dose for at least 6 days.

FIG. 15.—Alternate-day injection of 0.05 mg. thyroxin from second to thirtieth days. Posterior and anterior breast feathers are normal in appearance. Back and saddle have barbulation and melanin deposition in excess of normal.

PLATE I

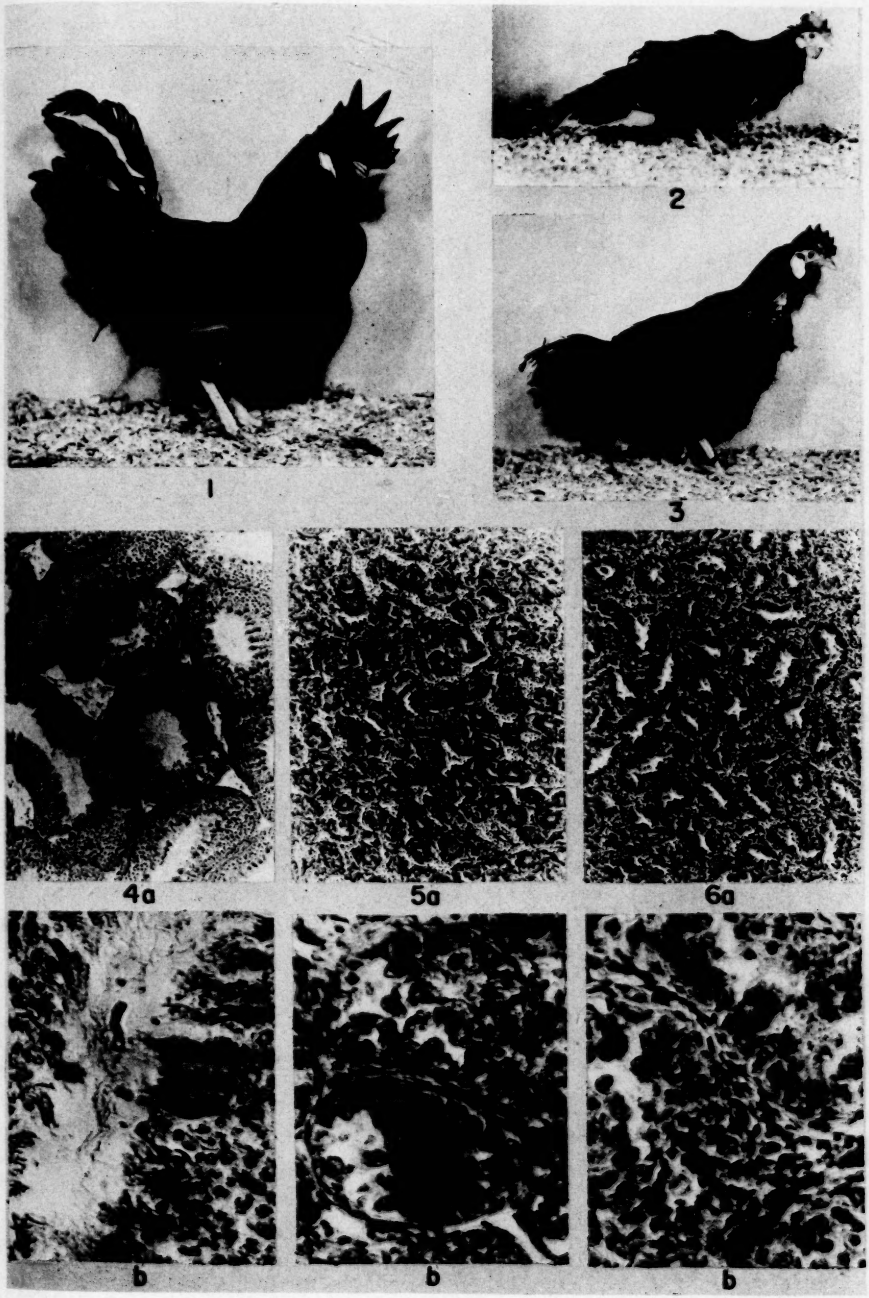


PLATE II

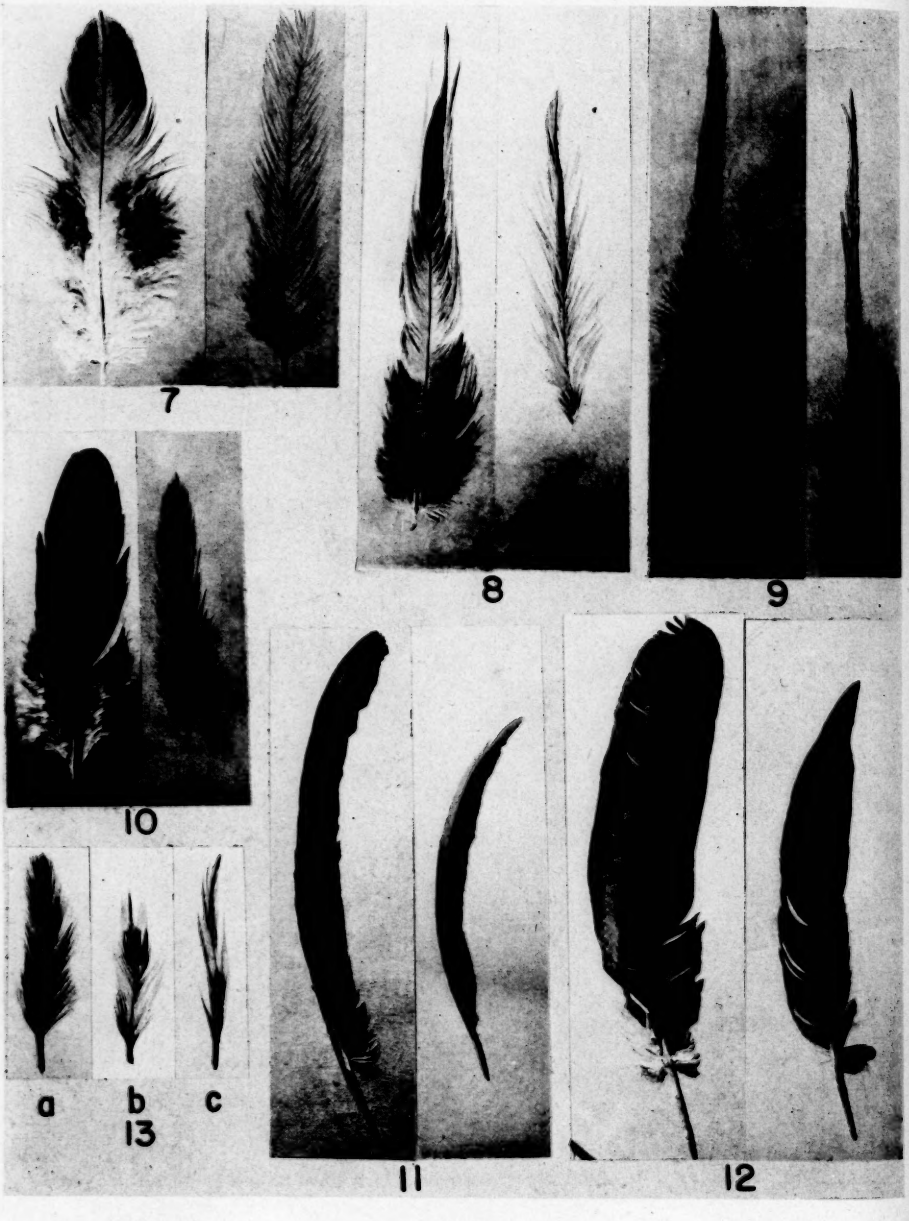
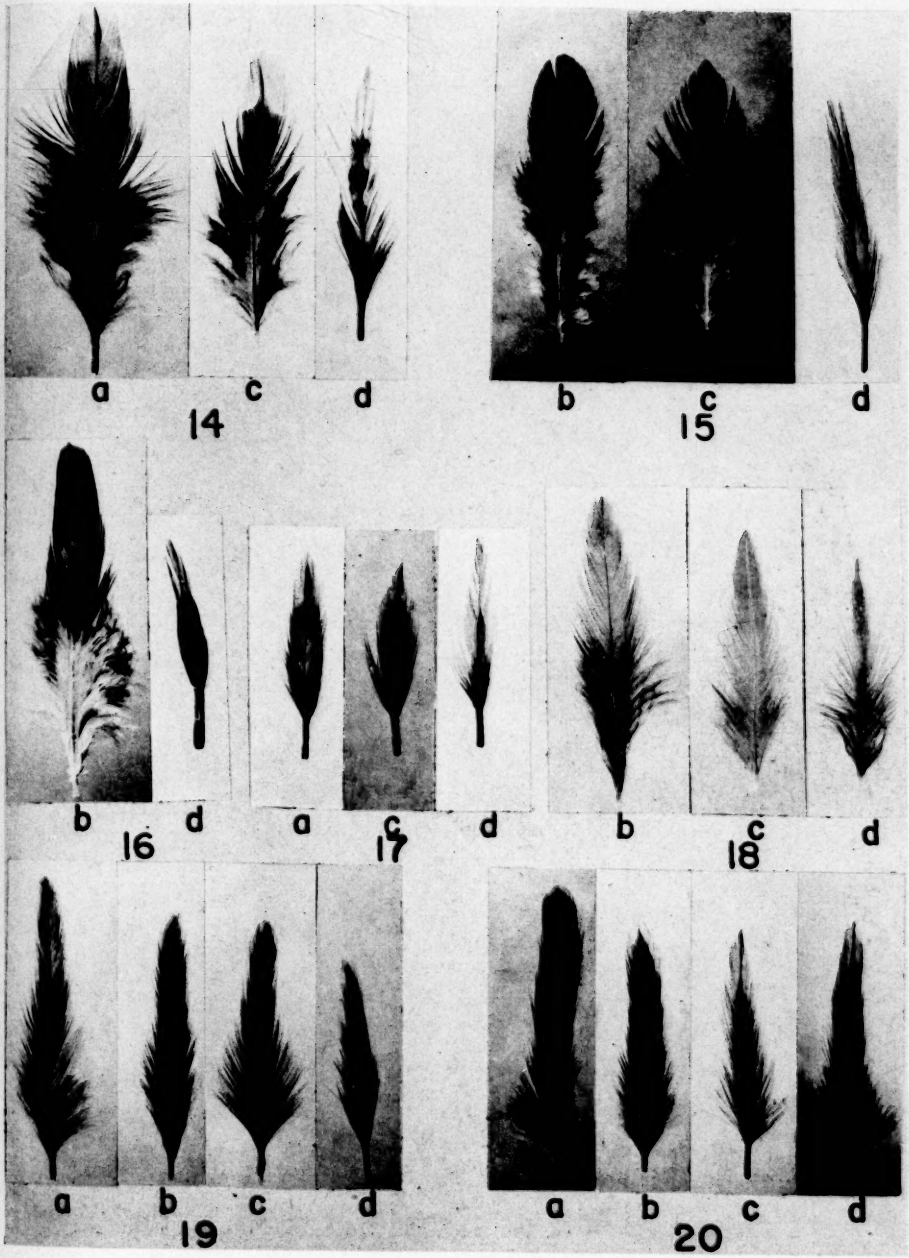


PLATE III



FIGS. 16–20.—Photographs of feathers of adult normal and thyroidectomized roosters after administration of alpha-estradiol benzoate. (In all figures, *a*, posterior breast; *b*, anterior breast; *c*, back; *d*, saddle.) Note the athyroidic condition of the feather tips in Figs. 17–20.

FIG. 16.—Daily administration to normal rooster of 13 γ of estradiol for 2 days, starting on day 14. Salmon-colored area develops in breast, and mottled dark brown and broad webbed area forms in back and saddle.

FIG. 17.—In athyroidic rooster, daily administration of 30 γ of estradiol for 15 days, starting on day 18 or 22. Breast shows slight broadening of web. Saddle shows broadening of web and deposition of black melanin in a mottled pattern.

FIG. 18.—Daily administration in an athyroidic rooster of 109 γ of estradiol for 20 days, starting on day of plucking or 4 days later. Breast feathers show a broadening of vane and web and a deposition of black melanin in a mottled pattern. Back displays a broader vane and web and a mottled deposition of black and brown melanin.

FIG. 19.—Daily administration in an athyroidic rooster of 194 γ for 22 days, starting on day of plucking or 4 days later. All areas show a broadening of web and vane and deposition of black and brown melanin in a mottled pattern.

FIG. 20.—Group I incompletely thyroidectomized rooster received 75 γ of estradiol daily for 8 days, starting on day 18, followed by 125 γ for 10 days. Greater degree of barbulation and black and brown melanin deposition in all areas than with larger doses in athyroidic roosters.

NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

BIOLOGICAL ACTIONS OF SEX HORMONES,[1] BY HAROLD BURROWS

Reviewed by C. DONNELL TURNER
Northwestern University

THIS volume by Burrows is a coordinated summary of the sex hormones and their biological actions within the organism. It must be appreciated that it is an extremely difficult undertaking to present in readable form a résumé of what is known about a subject in which current researches are as varied and numerous as they are in the field of sex physiology. Moreover, since biologists are concerned with changing processes within living organisms, they are often deprived of quantitative data and are forced to describe in detail the specific conditions under which their qualitative observations were made. Considering that these difficulties contribute to diffuseness in a narrative, it is the reviewer's belief that the author of this volume has succeeded in presenting the essentials of the subject in a fairly direct and comprehensive manner. This is not a "popular book on sex," but it is intelligible to readers having a moderate understanding of biological principles and terminology.

Since the war interrupted much good work in this field and hampered the dissemination of information, it is likely that inadequate consideration has been given to recent researches emanating from foreign countries. This is no criticism of the book, for it is probable that the quantity and quality of such work has not been sufficient to alter the general picture.

For convenience, all references to the literature, well over two thousand in number, are cited in the text and printed alphabetically at the end of the volume. This thorough documentation should make the volume extremely valuable to those who wish to refer to the original publications in particular fields.

There are instances in which the terminology employed in the book may be a bit confusing to American workers. For example, the pituitary gonadotropin is referred to as "FRH" (follicle-ripening hormone) instead of "FSH" (follicle-stimulating hormone) as it is usually designated in this country. Moreover, the term "ICSH" (interstitial-cell–stimulating hormone), introduced by Evans and co-workers when they thought that the factor which stimulates the interstitial cells was not the factor which produces luteinization, continues to be used, even though most workers now agree that the terms "LH" (luteinizing hormone) and "ICSH" are synonymous and refer to the same substance.

Part I contains four chapters dealing with the gonadotropins of hypophyseal and placental origins. The sources of these substances are discussed and their functions considered with reference to

[1] Cambridge: At the University Press; New York: Macmillan Co., 1945. Pp. x+514. $8.50.

hormonal balance and sexual activity. Three of the chapters elucidate the varied environmental and organismic factors which influence the synthesis and release of the gonadotropins and the factors which condition the reactivity of the gonads to them.

The single chapter of Part II presents a general view of the gonadal hormones and includes such topics as chemical structure, functional influences, gradients of responsiveness, and reversible and irreversible effects. Attention is directed to the fact that the terms "male hormone" and "female hormone," introduced through necessity by earlier endocrinologists, are misleading and inappropriate in the light of present knowledge. It is no longer possible to regard estogens and androgens as originating only from ovaries and testes, respectively, and as acting solely upon the organs of one sex. Since neither type of hormone is peculiar in origin or effect, the terms "male" and "female" hormones convey false and deceptive connotations which are not conducive to straightforward thinking.

The five chapters constituting Part III are devoted to the androgens. The actions of these substances upon the gonads, sex accessories, and secondary sex characters of both sexes are discussed at length. One chapter is devoted to the influence of androgens upon tissues and organs other than those constituting the reproductive systems, e.g., adrenal, kidney, liver, pancreas, thyroid, parathyroid, thymus, salivary glands, integument, skeleton, and musculature.

Part IV contains ten chapters dealing with the biological actions of estrogens. The great mass of literature elucidating the actions of these materials upon the genitalia of both sexes is brought together in logical form, and the salient features are given their proper emphasis. The roles of genetic and endocrine factors in the etiology of mammary cancer are discussed.

The sources and functions of the progestins are considered in the two chapters which constitute Part V. The participation of the corpus luteum hormone in the regulation of cyclical phenomena in the female is emphasized.

Part VI contains one chapter, which is devoted to the cortical steroids having sexual functions. The pituitary-adrenal and adrenal-gonadal relationships are summarized as adequately as present knowledge permits. Since there has not yet been time for repetition of experiments and the re-evaluation of the evidence, many of the concepts presented in this chapter necessarily remain uncertain.

This volume is a timely contribution to sex physiology and should be of inestimable worth to those already engaged in research therein or to those who are casting about for a promising field of investigation.

THE DIRECTIVENESS OF ORGANIC ACTIVITIES,[1] BY E. S. RUSSELL

Reviewed by W. C. ALLEE
University of Chicago

IN THE Preface E. S. Russell states that this small essay represents an adventure in biological thought, centering about a rejection of the mechanistic approach to biology. He continues: "The living thing *can* be treated as a physicochemical system or mechanism of great complexity . . . . but such an approach leaves out of account all that is distinctive of life . . . . and completely disregards its psychological aspect." He adds that in this book he has kept clear of philosophical problems so far as possible and has based his case on recorded facts relating chiefly to animals. Incidentally, the "facts" are carefully selected, and the author turns to something much like pure philosophy in the final pages.

An organism is recognized as being almost purposive, but "the neutral word *directive*" is selected (p. 6) as being in keeping with the "simple objective point of view" that the author says is characteristic of this book. The directive activity shown by individuals "distinguishes living things from inanimate objects" and furnishes a general characteristic of all organic activity. Russell leans strongly toward a teleological approach (p. 8). It is the goal ahead rather than innumerable past repetitions that explains present-day life. Physicochemical analyses lead to the acquisition of masses of unrelated facts of biochemistry and biophysics but not to an understanding of the cardinal principles, laws, and concepts of functional biology (p. 9).

Examples are drawn from many phases of biology, especially of animal biology, and include, among others, (1) healing of simple wounds; (2) repair of caddis-worm cases; (3) collection of functioning nematocysts by *Microstoma;* (4) control of the number of erythrocytes in mammalian blood; (5) hypertrophy and atrophy of liver and kidneys; (6) regulation of body temperature; (7) fitting the oxygen need, within limits, to the available supply; (8) similar regulation concerning water; and (9) food. Cell lineage, regeneration of lost parts, and various kinds of goal-directed activity at the behavior level—all are used to give supporting evidence. In a living animal, disturbed relations result in changed structural and functional patterns; these are considered directive, in that they remedy the deficiency or help establish a new norm that will fit the changed conditions (pp. 44 and 77).

Directiveness, as shown by living organisms, is related to creativeness; and by "creativeness" Russell means (p. 148) "the amazing power which living organisms possess of building up from simple beginnings, through their own activity, elaborate functional organizations of extreme complexity and adaptability." The various harmonies of cellular structures of plants (p. 152), of sponge anatomy (pp. 158 ff.), and of bones (p. 165) are used to illustrate the point. The author maintains (p. 171) that these cannot "be interpreted solely in terms of physical and chemical laws." Although in the early part of the book there is a clear statement that neither the mechanistic nor the vitalistic theory of life is satisfactory (p. 1), by the last pages the emphasis has shifted quite away from the mechanistic side, without, however, def-

[1] Cambridge: At the University Press, 1945. Pp. 196+23 figs. $2.00.

initely embracing vitalism. There is little recognition of evolutionary development; in fact, time as a major dimension plays little part in the present book, aside from its role in ontogeny. Near the end (p. 180) we find a brief recognition of the mnemic factor(s) in which there is a "striving to repeat, to reproduce the pattern common to the species . . . . impressed upon it by countless repetitions of essentially the same course of activity."

Reading this compactly written small book, I was reminded of a dinner conversation with two distinguished colleagues. The embryologist developed the theme that to understand cleavage and other developmental events it is necessary to keep the final result in mind, since the existing processes do not reveal their true significance unless viewed as forerunners of a meaningful end. The general physiologist, who had heard this opinion before from the same source, commented in a tired, patient voice that these events recur generation after generation. Such an approach is basically sound. The apparent units are integral parts of a sequence that is inherited as a whole rather than as fragments, and the whole has survival values that have been much tested by natural selection.

The present volume is a work of art in planning and execution. All the points made have an element of truth, usually a very large element; but I am not sure of the truth of the composite mosaic. The citations from many sources tend to carry conviction until one reflects on the work that is not cited. Before completely rejecting physicochemical explanations in biology, we need to remember that the physics and chemistry of many matters are incompletely known as yet. The centuries-old appeal to deferred judgment pending further evidence still has validity, and the possibility—even the probability—of advances in basic knowledge is high. It takes more courage to be a modern mechanist than to be a modern vitalist; to be an emergent evolutionist is an easier solution, and Russell's position as a plain functionalist is still more easily defended, although it avoids even attempting to make fundamental explanations. In the last resort it is necessary to remember this partial solution, while keeping one's mind open to other possibilities, many of which are still unknown, even in these early months of the atomic age.

EMBRYOLOGIE CHIMIQUE[1] BY JEAN BRACHET

Reviewed by L. G. BARTH
Columbia University

BRACHET addresses this book to all biologists, hoping to attract some of them to the field of chemical embryology. He has tried to present the researches so that they may be understood by the general biologist, yet he emphasizes that a broad scientific background is necessary to evaluate the facts from a critical viewpoint. This critical attitude is maintained throughout the analytical work, so that the reader is put on guard and does not readily accept the claims made by investigators. However, Brachet realizes the need for guiding hypotheses and in the conclusion of several chapters offers tentative and cautious interpretations, hoping that these will serve as points of departure for new researches.

The organization of the book is differ-

[1] Paris: Masson et Cie, 1944. Pp. 509 + 127 figs. Fr. 420.

ent from that of Needham's treatise on chemical embryology, as Brachet prefers to present the chemical investigations in the classical form of experimental embryology, beginning with the study of the gametes, passing on to fertilization and cell division, and ending with the problems of differentiation and regeneration. A concluding chapter sums up the facts acquired, controversial points, and perspectives for the future. Here he closes with optimism:

> L'heure approche où elle [embryologie chimique] nous fera saiser la signification profonde des gradients, des champs, des potentialités que l'embryologie expérimentale nous a fait connaître; mais dès maintenant l'embryologie chimique apporte des contributions importantes à la solution des énigmes de la morphogénèse et, à ce titre, elle s'intégre pleinement dans le vaste cadre de l'embryologie causale, telle que l'a conçue Albert Brachet.

In the first chapter on problems and methods there is an excellent discussion of the use of histochemical methods for detection of compounds in sections and a treatment of ultra-microchemical methods. In a valuable chapter on formation of gametes Brachet has brought together in a critical fashion what is known of the distribution of enzymes and substrates in the egg and spermatozoön. Under biochemistry of fertilization Brachet discusses the nature of sperm extracts and egg extracts, drawing largely on the work of Hartmann, Kuhn, Schartau, and Wallenfels for a clarification of the number of substances involved and their activity. Continuing with the changes in oxygen consumption at fertilization, he places the emphasis on the sea-urchin egg. Here Brachet accepts Ballentine's interpretation of the increase in respiration and questions Örstrom's failure to detect changes in reducing power. Örstrom's work is treated in detail, with a valuable summary of the various chemical changes that accompany fertilization.

The role of the calcium ion in fertilization according to Heilbrunn and Dalcq is discussed in some detail. Brachet concludes that the general hypothesis of release of calcium is well founded and that it probably will tie up with the change in the state of the proteins. He reviews the theories of Loeb, R. S. Lillie, F. R. Lillie, Bataillon, Dalcq, and Heilbrunn, concluding that these diverse theories soon should form a single complete theory of activation of the egg.

Brachet's chapter on metabolism and cell division brings together the inconclusive data regarding variations in respiration during mitosis. Building upon Rapkine's hypothesis that mitosis involves liberation of -SH groups through reversible denaturation of chromosomal proteins, he suggests a "biochemical" interpretation of mitosis patterned after the ATP-myosin system in muscle.

One of Brachet's special interests, and one which he treats with most intensity throughout the book, is the role of nucleic acids. The synthesis of thymonucleic acid may be a partial synthesis, in which ribonucleic acid is the source, or a total synthesis, in which a precursor of the purines is the source. This section of the book contains much of interest to the cytologist, since Brachet presents in detail Caspersson's hypotheses regarding the biochemical constitution of salivary-gland chromosomes, and the interconversions during mitosis of ribonucleic and thymonucleic acids and the protein constituents of the chromosomes. Brachet, impressed by the constant presence of nucleic acid in cells characterized by intense protein synthesis, is particularly concerned with the biochemical basis for the relation between nucleic acids and the synthesis of proteins.

The analysis of morphogenesis and metabolism in the sea-urchin egg has given rise to the gradient theories of

Runnström, Child, and Ranzi. Brachet severely criticizes all three theories and points to the work of Lindahl and Holter as showing, by direct measurements of oxygen consumption, no respiratory gradient. On the whole, Brachet concludes that, while the morphological differences in eggs are undisputed, the physicochemical differences underlying them are as yet unknown.

Brachet begins a discussion of the amphibian egg with studies using inhibitors such as NaF and iodoacetic acid. The results are not clear, and the question of permeability makes the interpretation difficult. The conclusion is that no simple relationship exists between the inhibitors and the metabolism of morphogenetic processes.

The utilization of glycogen and the production of lactic acid seem well established, but whether glycolysis is of the phosphorylating or of the nonphosphorylating type is controversial. However, the demonstration of triosephosphate dehydrogenase, coenzyme, and ATP would suggest a phosphorylating mechanism. On the whole, a small start has been made on general metabolism of the whole egg, but much more information is necessary and many new aspects must be examined before the significance of metabolism to developmental processes can be appreciated.

More significant to the problem of morphogenesis is the study of the metabolism of the parts of the egg. Studies of the metabolism of the whole egg are not easily related to a morphogenetic process, since so many processes, both physical and morphogenetic, are occurring simultaneously. Brachet does not confirm reports of Voss using histochemical methods that phospholipid is lost on invagination. That glycogen is lost on invagination is maintained by some investigators but not confirmed by others, with the weight of evidence in favor of some loss but with the correlation with induction unproved. Localization of ribonucleoproteins, both by basophilic staining and by determination of sulphhydryl groups released by trichloracetic acid, exhibits a dorsoventral gradient, a high concentration at the dorsal lip, and an anteroposterior gradient in the neural plate. Similar gradients demonstrated by Fischer and Hartwig and by Piepho favor Child's theory of gradients.

Direct respiration studies in explants are, however, controversial; and the work with the Cartesian diver, a very sensitive device, is unfortunately open to a very important criticism, which Brachet makes. Explants in the diver are in very small volumes of solution, and there is danger of the explants coming into contact with the surface film and thus cytolyzing completely or in part. Since the respiration of cytolyzed gastrula cells is about five times that of the living cells, the large variations, reaching 600 per cent in oxygen consumption, observed by Boell, Koch, and Needham, may be a result of cytolysis.

Brachet feels that the reviewer's findings on the animal-vegetal gradient of respiration associated with yolk content of the cells clarifies somewhat the contradictory measurements of O_2 consumption of the dorsal lip, as compared with explants from the opposite side of the egg. He concludes that there exist at the gastrula stage both quantitative and qualitative differences in the metabolism of parts.

Brachet's general review of the chemical nature of the organizer concludes with the evidence in favor of the hypothesis of an organizer released from the inactive state in noninductors by a variety of substances. Direct evidence is lacking, however, and Brachet goes on to make a strong case for ribonucleic acid as the

substance in tissues which stimulates neural plates, concluding that the action of dead tissues as organizers is mediated through nucleic acid.

With respect to the specificity of inductors, Brachet feels that the question is still open as to whether differences in inductors are quantitive or qualitative. Pointing out that the various gradient theories could account for differences in induction, he favors the proteins on a chemical basis as having the required specificity.

In the final chapter Brachet formulates a rather complete hypothesis for the action of the organizer and the formation of the neural tube. The action begins with the breakdown of ribonucleoproteins on invagination of the organizer. The resulting nucleotides diffuse into the presumptive neural plate and are resynthesized into new ribonucleoproteins. These then stimulate protein synthesis, giving rise to specific proteins for neuroblasts. Some of these proteins are of the fibrous type similar to myosin and are able to contract so as to cause the closure of the neural tube. Secondary effects are the stimulation of carbohydrate metabolism in the organizer by nucleotides and an increase in osmotic pressure and water content of the neural plate. (This latter has been disproved by specific-gravity measurement.)

The hypothesis is open to criticisms, but Brachet concludes:

> Pour stimuler son développement, nous avons, à maintes reprises, suggéré des expériences destinées à élucider des questions douteuses; nous aurons atteint notre but si l'intérêt d'un lecteur a été suffisamment éveillé pour qu'il éprouve le désir de contribuer à l'œuvre commune.

THE BIOLOGICAL BASIS OF INDIVIDUALITY,[1] BY LEO LOEB

Reviewed by R. W. GERARD
University of Chicago

THIS large volume, containing seven hundred pages of solid type almost unrelieved by tables or illustrations, is a summary of Dr. Loeb's life-work on individuality. It was provisionally written in 1930 and subsequent years, completed and revised between 1937 and 1945; and perhaps for this reason the thousand-odd selected references are overwhelmingly to work published in the 1920's or earlier.

The book is organized into eight main parts, dealing with organismal differentials from various aspects. The central theme is that each organism, as an entity, is to a degree differentiated from all others; and this individuality is manifested when the body fluids or cells of one organism are introduced into another. These individual-identifying properties of the body-as-a-whole are its organismal differentials. Such differentials are different from the organ or tissue differentials, which distinguish liver or kidney or epidermis in one individual, in that they somehow permeate the organism as a whole and are not just a mosaic of its part contributions. Organismal differentials are not, however, unitary. All members of a class or an order or a species, for example, are more similar to other members of the in-group than to members of an out-group; and the higher the level of group (class as against order or order as against genus), the greater the difference between the ins and the outs.

[1] Springfield, Ill.: Charles C. Thomas, 1945. Pp. xiv+714. $10.50.

These are the familiar serological and transplantation arguments as to phylogenetic relatedness.

Loeb's special emphasis is on the finer overtones of the theme, that even within the species a similar set of differentials exists between subspecies, races, family units, and single individuals. These individuality differentials—the most subtle and discriminating of the organismal ones—are revealed in the same way as the latter but require more refined analysis. The most sensitive index is the host reaction (lymphocyte response) to implanted tissue. This is minimal when bits are replaced in the same individual (autogenous transplantations), greater when tissue is moved to a sib or other individual of like family origin (syngenesious transplantations), and relatively severe when the transfer is between more unrelated individuals of a species (homeogenous transplantations).

The differentials are examined, in the main parts of the book, in relation to their analysis by the tissue-transplant method; their phylogenetic and ontogenetic development; their importance in cell interactions; their relation to tumors; their contribution to specific tissue reactions; their role as antigens; their relation to evolution; and their contribution to individuality at the psychological-social level.

The sweep of the author's concepts is stimulating and impressive, and the massed facts supporting them are a valuable source of information for readers of many interests. But the going is far from easy, for the writing is wordy and repetitious and the presentation fails to make a desirably sharp distinction between observed phenomena and their interpretation in Loeb's intellectual schema. There is much gold in this book—if the reader is willing to go through his own mining processes.